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ORIGINAL RESEARCH

Comparison of the Impact of tNGS with mNGS on Antimicrobial Management in Patients with LRTIs: A Multicenter Retrospective Cohort Study

Dan Li[1–3,*], Qingling Li[4,*], Zhen Huang[2], Wenhao Wu[2], Xinyuan Fan[2], Jing Liu[2], Ruoran Li[2], Qi Zhang[2], Xin Su[1]

[1]Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital, Clinical College of Nanjing Medical University, Nanjing, Jiangsu, 210000, People's Republic of China; [2]Department of Respiratory and Critical Care Medicine, Xuzhou Central Hospital, Xuzhou, Jiangsu, 221000, People's Republic of China; [3]Department of Respiratory and Critical Care Medicine, Jinling Hospital, Nanjing Medical University, Nanjing, 210002, People's Republic of China; [4]Department of Respiratory and Critical Care Medicine, Xuzhou First People's Hospital, The Affiliated Xuzhou Municipal Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, 221000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xin Su, Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital, Clinical College of Nanjing Medical University, Nanjing, Jiangsu, 210000, People's Republic of China, Email suxinjs@163.com; Qi Zhang, Department of Respiratory and Critical Care Medicine, Xuzhou Central Hospital, Xuzhou, Jiangsu, 221000, People's Republic of China, Email zhangqi1107@126.com

Background: tNGS and mNGS are valuable tools for diagnosing pathogens in lower respiratory tract infections (LRTIs), which subsequently influence treatment strategies. However, the impact of tNGS and mNGS on antimicrobial stewardship in patients with LRTIs remains unclear.

Methods: Patients diagnosed with LRTIs who underwent tNGS or mNGS between June 2021 and January 2024 were included. Patients who underwent both tNGS and conventional microbiologic tests (CMTs) were grouped into the tNGS group, the others were divided into the mNGS group. Then, the diagnostic efficacy of tNGS and mNGS was compared, along with their impact on antimicrobial management and clinical outcomes.

Results: 548 patients with an initial diagnosis of LRTIs who underwent tNGS or mNGS were evaluated. Finally, 321 patients were analyzed, with 117 patients in tNGS group and 204 patients in mNGS group. The overall pathogen detection rates for tNGS and mNGS were 89.74% and 89.71% (P=0.991). The distribution of detected pathogens was similar between tNGS and mNGS, with bacteria being the predominant microorganisms. The proportions of patients who underwent antimicrobial agent changes and received targeted therapy were not significantly different between tNGS and mNGS groups (P=0.270; P=0.893). Additionally, no significant differences were noted in the rates of antibiotic de-escalation, escalation, or changes in the opposite direction (all P>0.05). The same results was observed in the proportions of patients with addition or reductions in antiviral, antifungal, and antibacterial agents (all P>0.05). Hospital stays, improvement rate and mortality rate were also similar (all P>0.05).

Conclusion: tNGS and mNGS demonstrate comparable overall pathogen yield rates in patients with LRTIs. Furthermore, tNGS is also comparable to mNGS in terms of adjusting antimicrobial treatments and clinical outcomes, tNGS meets the clinical needs of most patients with LRTIs and can be firstly used for these patients.

Keywords: tNGS, mNGS, antimicrobial management, lower respiratory tract infection

Introduction

Respiratory infectious diseases remain a significant global health threat, both before and after the COVID-19 pandemic.[1] Lower respiratory tract infections (LRTIs) stand out as the primary cause of mortality among infectious diseases, contributing to over 2.49 million deaths worldwide in 2019.[2] Timely and accurate pathogen diagnosis in LRTIs is crucial for reducing the overuse of antimicrobial agents and the development of drug resistance, particularly in critically ill patients,[3] which in turn helps to lower healthcare-associated costs.[4] However, in China, approximately 40%-70% of

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patients with LRTIs receive empirical therapy without pathogen identification after conventional clinical microbiologic tests.[5,6] Similarly, in the United States, fewer than 50% of patients with community-acquired pneumonia (CAP) receive a microbiologic diagnosis and targeted treatment.[7]

Metagenomic next-generation sequencing (mNGS) is an advanced high-throughput sequencing technology capable of theoretically detecting almost all pathogens within a sample, including complex, rare, novel, and atypical etiologies.[8–10] In the diagnosis of fungal infections, most studies have shown that bronchoalveolar lavage fluid (BALF) mNGS has superior accuracy in diagnosing invasive pulmonary aspergillosis compared to traditional methods such as smears, culture, serum galactomannan (GM), and BALF GM.[11,12] However, mNGS is highly expensive and its results can be challenging to interpret. Targeted next-generation sequencing (tNGS) offers a partial solution to these limitations. tNGS combines next-generation sequencing with multiplex polymerase chain reaction (PCR) amplification or probe capture, which helps enrich target pathogens in patient samples and enhances the sensitivity of the test. The use of predefined panels in tNGS eliminates interference from human-derived genes, theoretically providing high sensitivity due to broad pathogen coverage. Additionally, tNGS improves detection throughput and reduces sequencing costs, as it requires only 80,000 reads—significantly fewer than the 20 million reads required by mNGS. Both tNGS and mNGS have been widely utilized in the diagnosis of LRTIs.[13–16] Moreover, a recent study found no significant difference in pathogen diagnostic efficiency between tNGS and mNGS in adults with pneumonia.[17] However, the differences in the clinical impact of tNGS and mNGS on LRTIs remain unclear. Hence, this retrospective cohort study was designed to compare the clinical antimicrobial management outcomes when adding tNGS or mNGS tests to conventional microbiologic tests (CMTs) in patients with LRTIs.

Methods

Study Design and Population

This multicenter retrospective cohort study included patients with LRTIs who were hospitalized at Xuzhou Central Hospital, Xuzhou First People's Hospital, and Nanjing Medical University Affiliated Jinling Hospital between June 2021 and January 2024. The inclusion criteria were: (1) age ≥18 years; (2) an initial diagnosis of LRTIs; and (3) tNGS or mNGS performed on sputum or bronchoalveolar lavage fluid (BALF). The exclusion criteria were: (1) incomplete clinical data; (2) NGS testing conducted on specimens other than BALF or sputum, such as nasopharyngeal swabs, blood, pleural fluid, or tissue samples; and (3) tNGS or mNGS results not available before the patient was discharged or died. The initial diagnosis of LRTIs was established based on specific criteria, including radiographic findings of new or progressive infiltrates, ground-glass opacities, consolidations, or interstitial changes, along with symptoms such as new-onset cough with sputum production or exacerbation of existing respiratory symptoms, presence of fever, lung consolidation signs, auscultatory abnormalities like altered breath sounds or localized rales, and peripheral blood white blood cell counts $>10\times10^9$/L or $<4\times10^9$/L. An initial diagnosis of LRTIs was established if criterion 1 was met along with any of the other criteria.[18–20]

Based on these criteria, researchers made a preliminary diagnosis of LRTIs, which was later confirmed by clinicians through the discharge diagnosis. In the process of clinical practice, the attending physician usually introduces the characteristics of these two detection methods to the patients or their family members. Then, patients decided the test because the NGS testing is totally self-paying. Patients who underwent both tNGS and CMTs were grouped into the tNGS group, while those who had mNGS and CMTs were in the mNGS group. The study adhered to the Declaration of Helsinki and received approval from the Medical Ethics Committee of Xuzhou Central Hospital (No. XZXY-LK -20240326-0042), and patient anonymity was maintained.

Microbiologic Methods and Data Collection

For the tNGS group, BALF or sputum samples were harvested and transported to one of four in vitro diagnostics laboratories in China (DIAN Diagnostics in Hangzhou; DINFECTOME in Nanjing; KingMed Diagnostics in Nanjing; DaAn Clinical Laboratory Center in Nanjing). BALF or sputum samples from the mNGS group were sent to one of six in vitro diagnostics laboratories in China (Matridx Biotechnology and DIAN Diagnostics in Hangzhou; KingMed

Diagnostics, Simcere Diagnostics, Practice Medicine, and TOPGEN in Nanjing). Each sample was subjected to either tNGS or mNGS for the identification of bacterial, fungal, mycobacterial, and viral pathogens. Additionally, CMTs were performed on all BALF or sputum samples in both groups. Briefly, the specimens were collected and sent to the microbiology laboratory of the local hospitals for staining and culture of bacteria, mycobacteria and fungi in alignment with the standard operating procedures. Acid-fast staining for *Mycobacteria*, Grocott-methenamine staining for *Pneumocystis jirovecii*, modified acid-fast bacilli staining for *Nocardia* were performed. Additionally, 1.3-β-D-glucan, galactomannan, and Cryptococcus capsular polysaccharide b antigen tests were performed for *Candida, Aspergillus*, and *Cryptococcus*, respectively. Xpert testing was done for *M. tuberculosis*. The clinicians decided to select the test items according to the situation of the patients.

For additional diagnostic assessments, blood specimens were utilized for both culture and detection of cryptococcal antigens, while urine samples facilitated tests for antigens of *Streptococcus pneumoniae* and *Legionella. Chlamydia pneumoniae, M. pneumoniae, L. pneumophila, influenza virus, Epstein-Barr virus, Cytomegalovirus, severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2*), and other herpes simplex viruses were tested by PCR assays and serological antibody detection. Fungal (1,3)-β-D-glucan, *Aspergillus* antigen and TB infection T cell using blood samples were also done. Tuberculosis purified protein derivative (PPD) test was performed by injecting 5 units of PPD subcutaneously into the middle and lower 1/3 of the volar forearm, then measured induration size after 72 hours. These additional tests were carried out based on clinical necessity as determined by the attending healthcare providers. The patients' diagnoses and clinical management were evaluated through medical chart reviews.

Definition and Outcomes

Clinical data were extracted from the electronic health records of the three hospitals involved in the study. According to a consensus about treatment of community-acquired pneumonia in immunocompromised adults, immunosuppression was characterized by one or more of the following: (1) active cancer or cancer within the past year relative to the lower respiratory tract infection, excluding patients with localized skin cancer or early-stage cancers (eg, stage 1 lung cancer); (2) inherited or genetic immunodeficiencies,; (3) receiving cancer chemotherapy; (4) HIV infection with a CD4 T-lymphocyte count <200 cells/uL or percentage <14%; (5) solid organ transplantation; (6) hematopoietic stem cell transplants; (7) receiving corticosteroid therapy with a dose ≥20mg prednisone or equivalent daily for ≥14 d or a cumulative dose >700 mg of prednisone; (8) receiving biological immune modulators; (9) receiving disease-modifying antirheumatic drugs or other immunosuppressive drugs (eg, cyclosporin, cyclophosphamide, hydroxychloroquine,methotrexate).[21] For the diagnosis of severe community-acquired pneumonia (SCAP), we adhered to the guidelines set by the Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS). SCAP was diagnosed if a patient met either one of two major criteria or at least three of the nine minor criteria. Major criteria included septic shock that necessitated the use of vasopressors and respiratory failure that required mechanical ventilation. The minor criteria were defined as a respiratory rate of 30 breaths per minute or more, a PaO_2/FiO_2 ratio of 250 or less, the presence of multilobar infiltrates, mental confusion or disorientation, a serum urea nitrogen level exceeding 20 mg/dl, a white blood cell count below 4000/μL or a platelet count below 100,000/μL, a core body temperature below 36°C, or hypotension that called for aggressive fluid resuscitation.[22]

Results from tNGS and mNGS testing were typically available within 1–2 days. As for CMT, the results from staining of bacteria, mycobacteria and fungi, testing of atypical pathogens and viruses by PCR assays and serological antibody detection usually returned within 1 day. The results of fungal (1,3)-β-D-glucan, Aspergillus antigen and TB infection T cell generally needed 2 days. PPD test result can be obtained after 3 days. While, the culture results of bacteria and fungi needed 4–5 days. The microorganisms detected usually needed to be determined in combination with their pathogenicity, host status and response to treatment, which was usually determined by the attending physician and reflected in the treatment and diagnosis. Colonized microbes were excluded based on established criteria.[23–28] For example, the 2016 IDSA Clinical Practice Guidelines for Candida Management stated that detection of candida in respiratory secretions usually indicated colonization. Therefore, unless candida was detected in the blood, we usually considered the detection of candida as a colonizing organism.[27] The diagnosis of NTM lung disease should be determined by combining clinical, radiologic and microbiological factors. If the diagnostic criteria are not met, it is

considered as colonization or contamination according to ATS/ERS/ESCMID/IDSA Clinical Practice Guideline.[28] We compared the proportion of patients with antibiotic changes between the tNGS and mNGS groups. In this study, we classified antibiotic changes into four categories according to a previous study:[29] (1) Antibiotic Escalation: the introduction of additional antibiotics or upgrading to more potent antimicrobial agents; (2) Antibiotic De-escalation: the reduction or downgrading of antibiotics from a broader to a more targeted spectrum; (3) Change in the Opposite Direction: alterations in antibiotic therapy that were counterproductive; (4) Unchanged: stable antibiotic regimens without any alterations in type or potency. Adjustments in antimicrobial strategies were assessed within the first week following the availability of the NGS results.

Additionally, we evaluated differences in the modification of additional antimicrobial treatments—including antiviral, antifungal, and antitubercular therapies—between the two study groups, with all outcomes assessed within a week of sampling. The treatment course for non-severe community-acquired pneumonia (NSCAP) in this study generally followed the guidelines of the Chinese Thoracic Society, lasting 5–7 days.[30] Treatment durations were extended for patients with severe CAP or those experiencing complex clinical scenarios. Additionally, we analyzed clinical parameters such as length of stay, improvement rate, and death rate.

Statistical Analysis

Categorical data were detailed as frequency distributions, while continuous data were summarized as means with standard deviations or as medians with interquartile ranges (IQRs), depending on their distribution. Differences between groups were analyzed using Student's *t*-test, chi-square (χ^2) test, or Mann–Whitney *U*-test, based on the data type and distribution. Subgroup analyses were also conducted. All statistical analyses were done utilizing SPSS 27.0 software, and differences were deemed statistically significant if $P < 0.05$ (two-tailed). All the graphs were drawn using GraphPad Prism 10.

Results

Initially, 548 individuals who received either tNGS or mNGS testing for LRTIs were recruited. These participants were initially identified with conditions such as pulmonary infections, pneumonia, chronic obstructive pulmonary disease, bronchiectasis, or lung abscesses. Exclusions were made for 29 patients younger than 18 years, 71 patients eventually found to have non-infectious conditions, 33 with incomplete medical data, 14 with duplicate entries, and 80 who had undergone NGS testing on non-respiratory samples like tissue, blood, or pleural fluid. Consequently, 117 patients were assigned to the tNGS group and 204 to the mNGS group (Figure 1). Analysis of the initial data showed no significant differences in baseline characteristics between the two groups, detailed in Table 1.

In the comparative analysis between the two groups, the mNGS group had a higher proportion of male patients (73.5% [150 of 204]) than the tNGS group (64.1% [75 of 117]) (*P*=0.076). Fewer patients in the mNGS group had neurological diseases compared to the tNGS group (12.7% [26 of 204] vs 19.65% [23 of 117], respectively; *P*=0.097). The procalcitonin (PCT) levels were lower in the mNGS group compared to the tNGS group (0.059 vs 0.102; *P*=0.091). The creatinine (Cr) levels were lower in the tNGS group compared to the mNGS group (57 vs 60.1; *P*=0.081). Despite these differences, none reached statistical significance.

Comparison of Pathogens Detection Between the tNGS and mNGS Groups

The overall pathogen yield rates for tNGS and mNGS were 89.74% (105/117) and 89.71% (183/204), respectively, with no significant difference between the two groups (*P*=0.991). tNGS detected a total of 161 pathogenic microorganisms, including 72 bacteria, 54 fungi, and 33 viruses. The top three bacteria identified by tNGS were *Staphylococcus aureus* (14/8.7%), *Pseudomonas aeruginosa* (13/8.1%), and *Streptococcus pneumoniae* (10/6.2%). The top three fungi were *Aspergillus* (34/21.1%), *Pneumocystis jirovecii* (13/8.1%), and *Candida albicans* (9/5.6%). The top three viruses were *influenza A virus* (16/9.9%), SARS-CoV-2 (7/4.3%), and *Epstein-Barr virus* (6/3.7%). mNGS detected 233 microorganisms, including 143 bacteria, 65 fungi, and 20 viruses. The top three bacteria identified by mNGS were *Streptococcus pneumoniae* (28/12.1%), *Mycobacterium tuberculosis* (28/12.1%), and *Pseudomonas aeruginosa* (27/11.7%). The top three fungi were *Aspergillus* (49/21.2%), *Pneumocystis jirovecii* (14/6.1%), and *Candida albicans* (9/3.9%). The

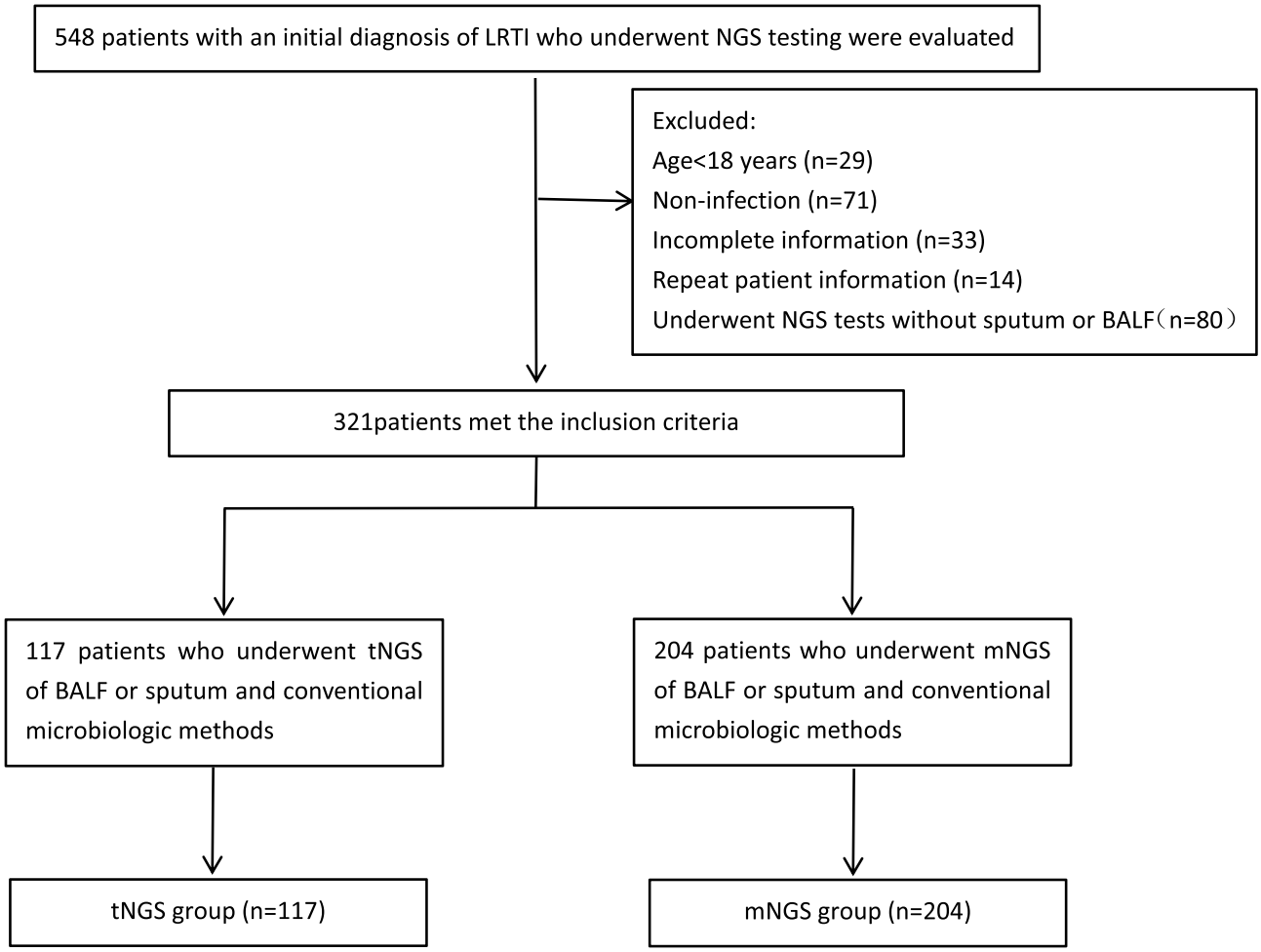

Figure 1 Flowchart of patients included in the study.
Abbreviations: LRTI, lower respiratory tract infection; tNGS, targeted next-generation sequencing; mNGS, metagenomic next-generation sequencing.

distribution of detected pathogens was similar between tNGS and mNGS, with bacteria being the predominant pathogens, followed by fungi and viruses (Figure 2a and b). The top 10 pathogens detected by tNGS and mNGS are shown in Figure 3a and b. Notably, *Aspergillus* was the most frequently detected pathogen in both tNGS and mNGS in this cohort study.

Comparison of Antimicrobial Changes Between the tNGS and mNGS Groups

The proportions of patients with changes in antimicrobial agents and those receiving targeted therapy were not significantly different between the tNGS and mNGS groups (79.48% [93 of 117] vs 74.02% [151 of 204], respectively; *P*=0.270; 54.7% [64 of 117] vs 53.9% [110 of 204], respectively; *P*=0.893). Additionally, no significant differences were noted between the two groups in the rates of antibiotic de-escalation (5.13% [6 of 117] vs 10.8% [22 of 204], respectively; *P*=0.084), escalation (61.54% [72 of 117] vs 50.5% [103 of 204], respectively; *P*=0.056), or changes in the opposite direction (9.4% [11 of 117] vs 10.29% [21 of 204], respectively; *P*=0.797). The proportion of patients with newly added antiviral agents was higher in the tNGS group than in the mNGS group (14.52% [17 of 117] vs 8.33% [17 of 204], respectively; *P*=0.083), though this difference was not statistically significant. Similarly, the proportions of patients receiving additional antifungal agents (36.75% [43 of 117] vs 31.37% [64 of 204], respectively; *P*=0.325) and antitubercular agents (8.55% [10 of 117] vs 13.73% [28 of 204], respectively; *P*=0.167) were comparable between the two groups. Regarding the reduction of antimicrobial agents, no significant differences were observed in the proportions of patients with reductions in antiviral agents (2.56% [3 of 117] vs 0% [0 of 204], respectively; *P*=0.090), antifungal

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Table 1 Baseline Characteristics in Patients with Lower Respiratory Tract Infections

Characteristics	Patients, No. (%)		*P* value
	tNGS Group (n = 117)	mNGS Group (n = 204)	
Male sex	75(64.10)	150(73.52)	0.076
Age, median (IQR), y	65(52–75)	63(53–72)	0.331
Sample type (sputum/BALF)	38/79	50/154	0.123
Comorbid conditions			
Cardiovascular disease	45(38.46)	70(34.31)	0.456
Respiratory disease	48(41.02)	82(40.20)	0.884
Nerve disease	23(19.65)	26(12.75)	0.097
Endocrine disease	19(16.24)	37(18.14)	0.666
Rheumatic disease	9(7.69)	13(6.37)	0.652
Renal disease	8(6.83)	6(2.94)	0.100
Cancer	16(13.68)	40(19.61)	0.178
Liver disease	5(4.27)	12(5.88)	0.536
respiratory failure	30(25.64)	38(18.63)	0.139
ICU lodging rate	13(11.11)	29(14.22)	0.427
Immunocompromised	29(24.79)	52(25.49)	0.889
Receiving corticosteroid therapy	4(3.42)	4(1.96)	0.324
Receiving immunosuppressive drugs	9(7.69)	13(6.37)	0.652
Cancers	15(12.82)	35(17.16)	0.302
HIV infection with CD4 lymphocyte count <14%	1(0.85)	0(0)	0.364
Laboratory results, median (IQR)			
Cell count, ×10^9/L			
RBCs	4.18(3.59–4.46)	4.12(3.60–4.43)	0.483
Hb(g/L)	123(109–137)	121(107–132)	0.298
WBCs	7.68(5.52–10.79)	7.37(5.53–11.24)	0.917
Neutrophils	5.46(3.58–8.69)	5.1(3.37–8.68)	0.908
Lymphocytes	1.18(0.76–1.60)	1.27(0.75–1.83)	0.405
Eosinophils	0.07 (0.005–0.160)	0.075 (0.013–0.17)	0.268
Basophilic granulocyte	0.02 (0.01–0.03)	0.02 (0.01–0.03)	0.161
Platelets	223(181–304)	232(182–312)	0.576
CRP, mg/L	29.75(6.53–74.84)	31.23(6.81–95.4)	0.731
PCT, ng/mL	0.102(0.032–0.395)	0.059(0.029–0.209)	0.091
ALT(u/L)	19(13.5–39.3)	19.6(12.47–35.75)	0.915
AST(u/L)	19.1(15–29)	20.6(16–30.7)	0.552
BUN(mmol/l)	5.55(4.0–6.86)	5.13(4.15–6.66)	0.517
Cr(umol/l)	57(49.5–68.7)	60.1(51–74)	0.081
ALB(g/L)	34.1 (29.50–39.3)	33.95 (30.1–38.17)	0.916
TBIL(umol/l)	10.3(8.1–13.8)	10(7–14)	0.446
Final diagnosis			
CAP	70(59.83)	132(64.71)	0.384
SCAP	13(11.11)	14(6.86)	0.187
NSCAP	57(48.72)	118(57.84)	0.152
HAP	4(3.42)	6(2.94)	>0.999
AECOPD	17(14.53)	19(9.31)	0.154
Bronchiectasis	13(11.11)	32(15.69)	0.256
Lung abscess	4(3.42)	7(3.43)	>0.999
Others	13(11.11)	14(6.86)	0.187

Note: Others include infective exacerbation of interstitial lung disease, bronchitis, and infective exacerbation of bronchial asthma.
Abbreviations: AECOPD, acute exacerbation of chronic obstructive pulmonary disease; CAP, community-acquired pneumonia; CRP, C-reactive protein; HAP, hospital-acquired pneumonia; HIV, human immunodeficiency virus; ICU, intensive care unit; IQR, interquartile range; PCT, procalcitonin; SCAP, severe CAP; WBCs, white blood cells.

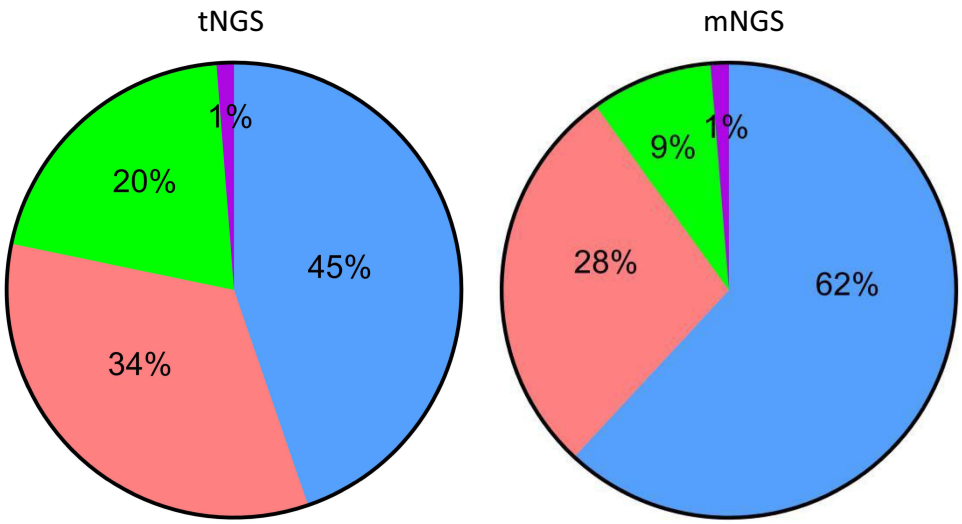

Figure 2 The distribution of pathogens detected by tNGS and mNGS.
Notes: Bacteria
Fungus
Virus
others

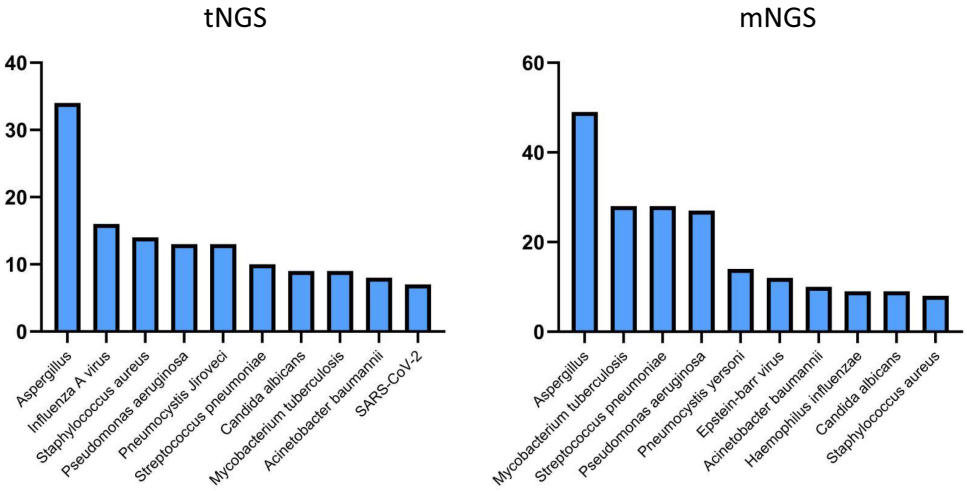

Figure 3 Top 10 pathogens detected by tNGS and mNGS.

agents (2.56% [3 of 117] vs 1.47% [3 of 204], respectively; P=0.789), or antibacterial agents (17.09% [20 of 117] vs 19.12% [39 of 204], respectively; *P*=0.652). Moreover, the rate of antibiotic exposure before sampling was similar between the tNGS and mNGS groups (92.31% [108 of 117] vs 88.24% [180 of 204], respectively; *P*=0.248). There was also no significant difference in the duration of antibiotic exposure before sampling (4 [1–7] vs 3 [1–6], respectively; *P*=0.694). All these data are presented in Table 2.

A subgroup analysis was conducted to evaluate the impact of tNGS and mNGS on antimicrobial adjustments in patients with community-acquired pneumonia (CAP) and in immunosuppressed patients. Among CAP patients, the proportion of those requiring the addition of antitubercular agents was lower in the tNGS group compared to the mNGS group (8.57% [6 of 70] vs 18.94% [25 of 132], respectively; *P*=0.052), though this difference was not statistically significant (Table 3). Meanwhile, in immunocompromised patients, a higher rate of escalation was identified in the tNGS group relative to the mNGS group (79.3% [23 of 29] vs 57.69% [30 of 52], respectively; *P*=0.050) (Table 4). Overall, the changes in antimicrobial agents between the tNGS and mNGS groups were similar in both CAP and immunosuppressed patients.

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Table 2 Use of Antimicrobial Agents in the tNGS and mNGS Groups

Variables	Patients, No. (%)		*P* value
	tNGS Group (n = 117)	mNGS Group (n = 204)	
Antibiotic therapy cases before sampling	108(92.31)	180(88.24)	0.248
Duration of antibiotic exposure before sampling, d	4(1–7)	3(1–6)	0.694
Targeted therapy	64(54.70)	110(53.92)	0.893
Antimicrobial agent change	93(79.49)	151(74.02)	0.27
Antibiotic change			
De-escalation	6(5.13)	22(10.78)	0.084
Escalation	72(61.54)	103(50.49)	0.056
Change in the opposite direction	11(9.40)	21(10.29)	0.797
Addition of other antimicrobial agents			
Antiviral agents	17(14.53)	17(8.33)	0.083
Antifungal agents	43(36.75)	64(31.37)	0.325
Antitubercular agents	10(8.55)	28(13.73)	0.167
Reduction of other antimicrobial agents			
Antiviral agents	3(2.56)	0(0)	0.09
Antifungal agents	3(2.56)	3(1.47)	0.789
Antibacterial agents	20(17.09)	39(19.12)	0.652

Table 3 Subgroup Analysis of Antimicrobial Agents in tNGS and mNGS Groups of CAP

Variables	Patients, No. (%)		*P* value
	tNGS Group (n =70)	mNGS Group (n =132)	
Antimicrobial agent change	54(77.14)	102(77.27)	0.983
Antibiotic change			
De-escalation	4(5.71)	17(12.88)	0.112
Escalation	42(60.0)	65(49.24)	0.145
Change in the opposite direction	5(7.14)	18(13.64)	0.167
Addition of other antimicrobial agents			
Antiviral agents	9(12.86)	11(8.33)	0.306
Antifungal agents	27(38.57)	44(33.33)	0.458
Antitubercular agents	6(8.57)	25(18.94)	0.052
Reduction of other antimicrobial agents			
Antiviral agents	2(2.86)	0(0.00)	0.119
Antifungal agents	1(1.43)	1(0.76)	>0.999
Antibacterial agents	12(17.14)	34(25.76)	0.165

Comparison of Clinical Outcomes Between the tNGS and mNGS Groups

The length of hospital stay was similar between the tNGS and mNGS groups (13 [10–19] vs 12 [9–16] days, respectively; P=0.051). Additionally, there were no significant differences in the improvement rate or death rate between the two groups (all $P > 0.05$) (Table 5).

Table 4 Subgroup Analysis of Antimicrobial Agents in tNGS and mNGS Groups of Immunocompromised Patients

Variable	Patients, No. (%)		*P* value
	tNGS Group (n =29)	mNGS Group (n =52)	
Antimicrobial agent change	26(89.66)	44(84.62)	0.526
Antibiotic change			
De-escalation	2(6.90)	8(15.38)	0.447
Escalation	23(79.31)	30(57.69)	0.050
Change in the opposite direction	1(3.45)	3(5.77)	>0.999
Addition of other antimicrobial agents			
Antiviral agents	7(24.14)	8(15.38)	0.331
Antifungal agents	12(41.38)	18(34.62)	0.546
Antitubercular agents	3(10.34)	6(11.54)	>0.999
Reduction of other antimicrobial agents			
Antiviral agents	1(3.45)	0(0.00)	0.358
Antifungal agents	0(0.00)	2(3.85)	0.535
Antibacterial agents	5(17.24)	9(17.31)	0.994

Table 5 Clinical Outcome in the tNGS and mNGS Groups

Variables	Patients, No. (%)		*P* value
	tNGS Group (n = 117)	mNGS Group (n = 204)	
length of stay, d	13(10–19)	12(9–16)	0.051
Improvement rate	96(82.05)	159(77.94)	0.381
Death rate	3(2.56)	6(2.94)	>0.99

Discussion

Our findings indicated that tNGS performed similarly to mNGS in the detection of pathogenic microorganisms in adults with LRTIs, aligning with findings from earlier studies.[17] Notably, tNGS detected more fungi and viruses than mNGS in this cohort. Despite these differences in detection capabilities, there was no significant disparity in the application of targeted therapies or the modification of antimicrobial treatments between the groups, firstly suggesting that tNGS is also comparable to mNGS in antimicrobial stewardship for patients with LRTIs. Additionally, the length of hospital stay, improvement rates, and death rates were similar between the tNGS and mNGS groups, demonstrating that clinical outcomes were not significantly different between the two methods.

mNGS has documented to be a valuable tool in diagnosing LRTIs, providing insights into etiological analysis, predicting drug resistance, and guiding antibiotic treatment, particularly in critically ill patients.[31–36] Compared to conventional microbiological tests, mNGS has shown a higher sensitivity, reaching 76.6%.[35] However, in patients with *Pneumocystis jirovecii* pneumonia, the diagnostic sensitivity and specificity of mNGS were comparable to those of PCR.[37] The positive rate of pathogen detection by mNGS varies widely, ranging from 69.69% to 95%, depending on factors such as region, season, and specimen type.[31,38–40] The distribution patterns of pathogens causing LRTIs detected by mNGS also differ among studies.[31,35,38] In recent years, tNGS has also been employed to diagnose pulmonary infections, investigate the epidemiology of LRTIs during influenza A virus pandemics, and detect drug-resistant *Mycobacterium tuberculosis*,[14,41,42] in both adults and children.[43,44] The overall positive rate of pathogens detected by tNGS in LRTIs was around 80% in a cohort of 167 patients.[41] Two studies directly comparing the utility of tNGS and mNGS in respiratory infections found that tNGS had similar performance characteristics to mNGS.[17,45] Our study also

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demonstrated that the positive detection rate of pathogens was similar between tNGS and mNGS. Notably, the highest number of pathogens are bacteria as shown in Figure 2a and b, but in Figure 3a and b, the most predominantly found pathogen is *Aspergillus*. The main reason for this phenomenon is that empiric antimicrobials therapy usually target bacteria. When empiric treatment fail, the detection rate of fungi increases. In our study, all patients enrolled had experienced treatment failure which increased the chance of fungal infection. In addition, the bacterial species detected were relatively dispersed leading the number of a particular type of bacteria is not particularly high. Among the detected fungus, candida was abundant, but according to the diagnostic criteria for candidiasis, most of the detected candida were judged to be upper respiratory infection or colonization in our study. Additionally, respiratory viruses can cause direct damage to the airway epithelium, hinder ciliary clearance and lead to local or systemic immune dysfunction or dysregulation, allowing *Aspergillus* to invade the tissue.[46] Previous studies have shown an association between viral respiratory infections and aspergillosis. *Aspergillosis* occurred in up to one-third of critically ill COVID-19,[47,48] and the incidence of influenza-associated pulmonary aspergillosis ranges from 7% to 30%.[49,50] Our research was in the midst of COVID-19 and the influenza pandemic, contributing to an increasing of secondary *Aspergillus* infections.Moreover, this result may also be influenced by the clinician's interpretation of next-generation sequencing results. In this study, tNGS also detected more influenza A virus cases. This may be attributed that tNGS tests both DNA and RNA, whereas most mNGS tests focused only on DNA, and the study coincided with the influenza season.

The use of mNGS has been shown in previous studies to help optimize antibiotic management. A multicenter prospective observational study demonstrated that mNGS led to a change in treatment in 37.1% of cases, including antibiotic de-escalation in 25.2% of pneumonia cases.[51] Another single-center retrospective study, which enrolled 140 patients with suspected LRTIs, found that antibiotic treatment was downgraded in 3.6% (5/140) of patients and upgraded in 23.6% (33/140) based on mNGS results.[52] After propensity score matching with a control group to balance the baseline characteristics of patients with LRTIs, Mengwei Yan et al found that adding mNGS detection to routine microbial testing could reduce the rate of antibiotic escalation.[36] Similarly, the impact of tNGS on antibiotic treatment has also been investigated. Zhenfeng Deng found that 38.8% (81/209) of enrolled pediatric and adult patients with pulmonary infections adjusted their treatment based on tNGS results from sputum samples.[14] However, no studies have directly compared the impact of these two next-generation sequencing tests on the adjustment of antimicrobial agents in patients with LRTIs. In the current study, we found no statistically significant differences between the tNGS and mNGS groups in terms of antibiotic escalation, antibiotic de-escalation, changes in the opposite direction, addition of antiviral agents, addition of antifungal agents, addition of antitubercular agents, or reduction of antiviral, antifungal, and antimicrobial agents. Similar results were observed in patients with CAP and in immunocompromised patients. These results were mainly due to the consistency of tNGS and mNGS in the detection of pathogens.These findings suggest that tNGS, when combined with traditional microbial assays, can meet most clinical needs in the treatment of LRTIs, similar to mNGS combined with conventional microbial tests. However, tNGS is significantly less expensive than mNGS, making it more accessible and easier to adopt. In our study, the rate of antimicrobial agent changes was 79.48% in the tNGS group, which is higher than the 38.8% reported in a previous study.[14] Similarly, the rate of antimicrobial agent changes in the mNGS group was 74.02%, also higher than the 27.2%-37.1% reported in earlier studies.[51,52] This discrepancy is likely because, in previous studies, antibiotic treatment adjustments were based solely on NGS results, whereas in our study, adjustments were made based on both NGS and CMT results. The rate of antibiotic changes in our study aligns closely with the 75.2% reported by Mengwei Yan, who also evaluated adjustments based on a combination of NGS and CMT results.

Hao et al reported that 89.12% of patients with LRTIs responded to mNGS-guided antibiotic adjustments, with 75.51% of patients showing a good prognosis. Clinical laboratory indicators, such as neutrophil count, C-reactive protein levels, and white blood cell count, declined significantly after treatment adjustments guided by mNGS results.[40] A retrospective study of eight patients with *Chlamydia psittaci* pneumonia found that tNGS was an economical and practical method for diagnosing this disease, leading to a good prognosis.[53] However, no studies have directly compared the impact of tNGS with mNGS on clinical outcomes in patients with LRTIs. In our study, The length of hospital stay in tNGS group was 13 days, which was similar with that of mNGS groups. The improvement rates were 82.05% and 77.94% in tNGS and mNGS group repectively, there was also no significant difference. These results are consistent with

those of the studies mentioned above.The death rate were similar between the two groups. These findings suggest that tNGS is comparable to mNGS in terms of prognosis, likely due to their similar diagnostic value for pathogen detection.

This retrospective analysis inherently carries biases related to data collection and interpretation. While efforts were made to equalize influencing factors across the tNGS and mNGS groups, undetected discrepancies could still undermine the robustness of our conclusions. Consequently, there is a compelling need for prospective studies to more accurately ascertain the clinical utility of tNGS and mNGS. Additionally, the testing results of tNGS and mNGS came from different institutions, and variations in detection capabilities and pathogen spectra among these institutions may impact the reliability of our findings. However, the three institutions involved in the study are medical associations, with regular academic exchanges and unified laboratory quality control. In addition, the results of tNGS and mNGS obtained from different institutions needed to be combined with CMT and clinical manifestations to guide the adjustment of antibiotics. So the differences in reagents and equipment from different institutions will not significantly affect our research results. Both sputum and BALF samples were tested using tNGS and mNGS. Although there was no difference in sample composition between the two groups, potential differences could still affect the interpretation of our results. Finally, this study is based on clinical data collected from three hospitals in Jiangsu Province, so the conclusions may not be entirely applicable to other regions.

In conclusion, our study identified no significant difference in the overall pathogen yield rate between tNGS and mNGS. Furthermore, tNGS was comparable to mNGS in adjustments of antimicrobial treatment when combined with conventional methods in patients with LRTIs. Lastly, no significant differences were noted in the clinical outcomes between the tNGS and mNGS groups. All these results indicating that tNGS was comparable to mNGS in clinical application. Considering that tNGS is much cheaper than mNGS and the return time is similar, we advise tNGS should be used firstly in lower respiratory infection. These conclusions needs to be further investigated through rigorously designed clinical studies.

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Disclosure

The authors report no conflicts of interest in this work.

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