Infection and Drug Resistance

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ORIGINAL RESEARCH

Metagenomic Next-Generation Sequencing of Bronchoalveolar Lavage Fluids Improves Pathogen Detection and Antimicrobial Stewardship in Lower Respiratory Tract Infections: A Retrospective Study

Xiangsong Ma[1], Qinqin Zhang[1,2], Xuelian Ji[1], Yun Xia[1], Ju Cao[1], Xiuyu Xu[1]

[1]Department of Laboratory Medicine, the First Affiliated Hospital of Chongqing Medical University, Chongqing, 400016, People's Republic of China; [2]Department of Laboratory Medicine, People's Hospital of Mingshan District, Ya'an, Sichuan, 625100, People's Republic of China

Correspondence: Xiuyu Xu, Department of Laboratory Medicine, the First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing, 400016, People's Republic of China, Email xuxiuyu85@126.com

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Purpose: With the advancement of metagenomic next-generation sequencing (mNGS), its role in diagnosing lower respiratory tract infections (LRTIs) has expanded rapidly. LRTIs remain a major global health burden, particularly in critically ill patients where diagnosis is challenging. Routine microbiological testing (RMT), including culture, microscopy, antigen detection, and PCR—are limited by low sensitivity, long turnaround times, and restricted pathogen coverage. This study assesses the diagnostic performance of mNGS in LRTIs, with emphasis on pathogen detection and resistance gene prediction, and compares it with traditional methods to clarify its clinical benefits and limitations.

Methods: This retrospective study included 367 hospitalized patients with suspected LRTIs. All patients underwent mNGS testing, which was compared with traditional diagnostic methods. We also used mNGS to explore the pathogen spectrum characteristics in critically ill patients with pneumonia and evaluated its applicability in predicting antimicrobial resistance genes and adjusting antibiotic treatment.

Results: For patients diagnosed with LRTIs, mNGS demonstrated superior microbial detection efficacy, particularly for bacteria and fungi, relative to culture (bacteria: 56.58% vs 17.37%, $P < 0.0001$; fungi: 49.65% vs 16.78%, $P < 0.0001$) and PCR (65.14% vs 45.14%, $P < 0.05$). In contrast to the non-severe pneumonia group, the detection rate of *Enterococcus faecium* was highest in the severe pneumonia group ($P < 0.001$), and the severe pneumonia group had more mixed infections ($P < 0.001$). In addition, mNGS showed high accuracy in predicting antibiotic resistance genes, with 90.57% agreement with antibiotic susceptibility testing (AST) results. Based on the mNGS results, 97.82% of patients underwent active adjustment to their antibiotic treatment regimen.

Conclusion: mNGS is an effective tool for diagnosing LRTIs, with significantly higher pathogen detection rates than traditional methods. mNGS also demonstrates high accuracy in predicting antimicrobial resistance, providing crucial support for clinical treatment decisions.

Keywords: LRTIs, mNGS, diagnosis, pathogens, antimicrobial resistance

Introduction

According to the Global Burden of Disease (GBD) study, lower respiratory tract infections (LRTIs) rank seventh among diseases causing mortality worldwide.[1] They pose significant health challenges, particularly in critically ill patients, in whom LRTIs can lead to severe complications, such as acute respiratory distress syndrome (ARDS), sepsis, and multiple organ failure. These complications substantially increase hospitalization and mortality risk.[2] LRTIs can be caused by bacterial, fungal, viral, or mixed infections. Correct identification of the causative pathogens is essential for proper

Received: 3 October 2025
Accepted: 9 December 2025
Published: 13 December 2025

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treatment, especially in the context of rising antimicrobial resistance.[3] Despite the use of various traditional screening methods, the etiology of LRTIs remains undetermined in a substantial proportion of patients.[4] Failure to promptly identify pathogens can result in prolonged treatment duration, poor prognosis, and even mortality.

Current laboratory diagnostic methods, including microscopy, culture, serological testing, and polymerase chain reaction (PCR), have limitations such as low sensitivity, long turnaround times, and narrow pathogen detection spectrum.[4,5] In addition, these methods may fail to detect specific pathogens because traditional culture methods depend on the specific growth conditions of pathogens, and some pathogens (eg, *Legionella, Mycobacteria*, and anaerobes) are challenging to grow in conventional media and environments. Serological testing relies on the production of specific antibodies, which may result in false-negative results during early infections. Moreover, PCR requires predefined primers and therefore cannot detect unknown or rare pathogens. In contrast, metagenomic next-generation sequencing (mNGS) provides a high-throughput, unbiased detection approach that comprehensively covers all potential pathogens and improves pathogen detection rates, particularly for atypical and hard-to-culture pathogens.[6] Furthermore, mNGS demonstrates superior performance in diagnosing pulmonary infections caused by mixed pathogens, with significantly higher sensitivity than traditional methods.[7] This treatment is particularly advantageous for patients with severe pneumonia, who often have critical conditions with mixed infections. Mixed infections can complicate antimicrobial selection, increasing the risk of antimicrobial resistant strains.[8] Moreover, mNGS is less affected by prior antimicrobial exposure compared to culture.[9] Given the growing challenge of antimicrobial resistance, mNGS enables faster identification of resistance genes, guiding appropriate treatment, improving the efficacy of anti-infective therapies, and reducing the overuse of broad-spectrum antimicrobial agents, thereby mitigating the development of antimicrobial resistance.

In this study, we retrospectively evaluated mNGS using bronchoalveolar lavage fluid (BALF) samples from 367 hospitalized patients with suspected LRTIs, including comparisons between severe and non-severe pneumonia groups. We analyzed pathogen detection rates, therapeutic impact, and resistance gene prediction relative to routine diagnostics. Despite the growing use of mNGS, its real-world influence on antimicrobial stewardship and resistance gene detection in LRTIs has not been well studied. This work aims to address this gap and provide comprehensive evidence for its clinical utility.

Methods

Study Patients

We retrospectively selected 445 patients admitted to the First Affiliated Hospital of Chongqing Medical University between December 1, 2022 and December 31, 2023, who were clinically diagnosed with suspected LRTI at admission. Only patients fulfilling diagnostic criteria for community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP), and who required bronchoalveolar lavage as part of routine evaluation, were eligible. Medical records were reviewed to ensure that no patients had non-infectious pulmonary conditions such as pulmonary embolism or cardiogenic pulmonary edema at the time of BALF sampling. A series of inclusion and exclusion criteria were established (Figure 1). After excluding 78 patients, 367 were ultimately included. Based on guidelines and a comprehensive evaluation by the hospital's expert team, 334 patients were diagnosed with LRTIs. According to the diagnostic criteria, 109 patients were ultimately diagnosed with severe pneumonia.[10,11] BALF samples from participants were collected for mNGS analysis and culture, with some undergoing PCR and other tests. Additional routine tests were performed as clinically indicated based on the patients' conditions.

BALF Preparation

According to clinical practice guidelines,[12] the procedure was performed by licensed physicians at our hospital who had undergone systematic training and were proficient in bronchoscopic diagnostic and therapeutic techniques. First, the bronchoscope's insertion tube was lubricated. A suitable nasal passage was selected for insertion, ensuring that the bronchoscope remained in a "neutral" position. For patients with hypertrophic turbinates, ephedrine was administered or an oral approach was used to prevent nasal mucosal damage. After entering the nasal cavity, negative suction was avoided. When the bronchoscope reached the glottis, local anesthesia was administered for surface numbing. Upon reaching the carina, additional local anesthesia was administered to relieve airway congestion. After advancing to the

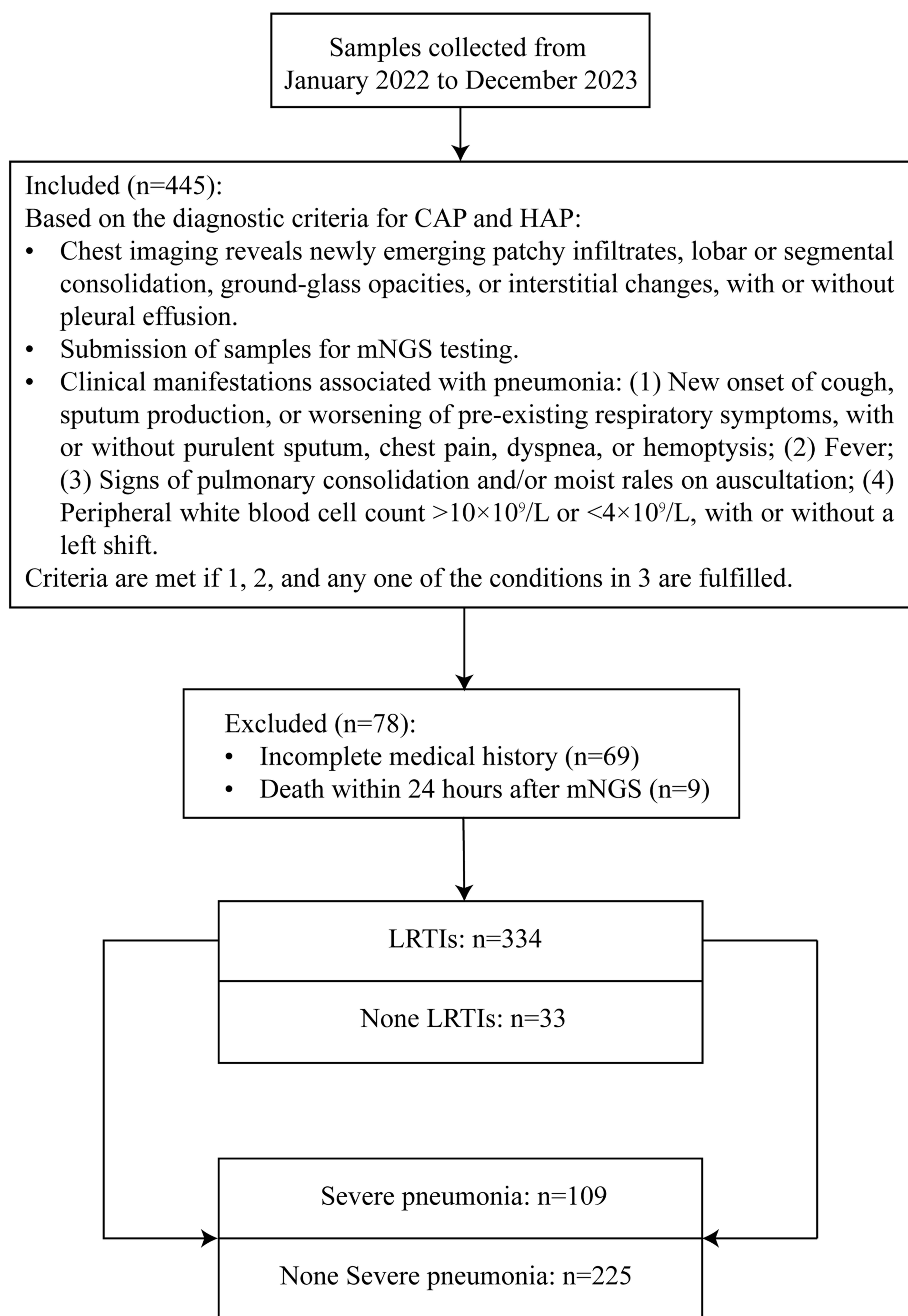

Figure 1 Flowchart of patient enrollment. Based on the inclusion and exclusion criteria, 367 cases were selected from 518 for analysis.
Abbreviations: LRTIs, lower respiratory tract infection diseases; non-LRTIs, non-lower respiratory tract infection diseases; mNGS, metagenomic next-generation sequencing.

target lung segment, another dose of local anesthesia was administered before lavage of the target lung segment. Sterile 0.9% saline was preheated to 37°C before the procedure. Using a syringe, saline was rapidly instilled through the bronchoscope's working channel in aliquots of 20–50 mL, with a routine lavage performed 3–5 times, for a total volume of 60–120 mL. Finally, more than 5 mL of lavage fluid was collected in a sterile container. One part of each sample was sent to the clinical microbiology laboratory for traditional microbiological testing, while the remaining BALF specimens were stored at 4°C for mNGS analysis.

Routine Microbiological Detection

Routine microbiological culture was performed using BALF samples inoculated onto blood, MacConkey, chocolate, or Sabouraud agar plates to culture common respiratory pathogens, including bacteria and fungi. Identification was conducted using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry (Biomérieux, Craponne, France). For samples from patients with suspected fungal infections, fungal culture and *Aspergillus* antigen testing (DANNA Bio, index ≥0.8 positive) were performed with BALF, while serum was used for 1,3-β-D-glucan testing (DANNA Bio, index >95 pg/mL suggestive of invasive fungal infection), *Aspergillus* antigen testing (DANNA Bio, index ≥0.5 positive), *Aspergillus* and *Candida* IgG antibody assays (DANNA Bio, index ≥120 AU/mL positive), and cryptococcal capsular antigen testing (DANNA Bio). For patients with high suspicion of tuberculosis infection, BALF specimens were tested using the Xpert MTB/RIF Ultra assay (Cepheid), which simultaneously detects Mycobacterium tuberculosis complex DNA and rifampicin resistance–associated mutations. In addition, some patients' BALF samples were analyzed using multiplex PCR-based rapid infectious disease testing (CrAg) (BAD Bio), which targeted pathogens including *Escherichia coli, Klebsiella pneumoniae, Streptococcus pneumoniae, Stenotrophomonas maltophilia, Haemophilus influenzae, Pseudomonas aeruginosa, Enterobacter cloacae, Staphylococcus epidermidis, Acinetobacter baumannii, Staphylococcus aureus, Moraxella catarrhalis, Serratia marcescens, Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia trachomatis, Bordetella pertussis, Candida albicans, Candida tropicalis, Candida glabrata, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, and Cryptococcus neoformans*. Oropharyngeal swabs were tested using nucleic acid amplification tests for influenza A and B viruses and other respiratory viruses, including respiratory syncytial virus, adenovirus, parainfluenza virus type I, and parainfluenza virus type III. Whole blood samples were subjected to real-time PCR to detect cytomegalovirus (CMV) (DAAN GENE) and Epstein–Barr virus (EBV) (SHENGXIANG Bi). To prevent bias associated with cross-sample comparisons, we clarify that the 1,3-β-D-glucan testing, *Aspergillus* antigen testing, and Aspergillus/Candida IgG were used solely as ancillary diagnostic tools and were not evaluated in a head-to-head manner against mNGS. Furthermore, several assays—including cryptococcal antigen (CrAg), CMV DNA PCR, and EBV DNA PCR—are technically validated and clinically implemented only on serum specimens at our center. Because these tests cannot be performed on BALF, serum-based results served as the only feasible clinical comparator when assessing their concordance with BALF mNGS findings.

mNGS Methodology

DNA and RNA Extraction and Library Construction

Pathogens and human cells were separated from 1 mL BALF samples by centrifuging it at 12,000 g for 5 min. The host nucleic acid was then removed from the precipitate using 1 U Benzonase (Sigma) and 0.5% Tween 20 (Sigma), which were incubated at 37°C for 5 min. A 400 µL dose of terminal buffer was then added to halt the reaction. A Minilys Personal TGrinder H24 Homogenizer (catalog number: OSE-TH-01, Tiangen, China) was used to beat beads after transferring a total of 600 µL mixture into new tubes containing 500 µL of ceramic beads. DNA was then extracted and eluted from 400 µL of pretreatment samples using a QIAamp UCP Pathogen Mini Kit in 60 µL elution buffer (catalog number: 50214, Qiagen, Germany). Using a Qubit dsDNA HS Assay Kit (catalog number: Q32854, Invitrogen, USA), the isolated DNA was quantified. Total RNA was extracted with a QIAamp® Viral RNA Kit (Qiagen) and ribosomal RNA was removed by a Ribo-Zero rRNA Removal Kit (Illumina). cDNA was generated using reverse transcriptase and dNTPs (Thermo Fisher). Libraries were constructed for the DNA and cDNA samples using the KAPA low throughput library construction kit (KAPA Biosystems, USA) following the manufacturer's instructions. Library was quality assessed by Qubit dsDNA HS Assay kit followed by High Sensitivity DNA kit (Agilent) on an Agilent 2100 Bioanalyzer. Library pools were then loaded onto an Illumina Nextseq CN500 sequencer for 75 cycles of single-end sequencing to generate approximately 20 million reads for each library.

Interpretation of the mNGS Data

The suspected pathogens were identified after removing common background microorganisms and contaminants compared to the negative test controls (NTCs). We analyzed the genus-level relative abundance, species-specific read number (SSRN), genome coverage (%), depth, and species-specific reads per million (RPM) ratio. A virus was considered positively detected if SSRN ≥ 3 covering at least three non-overlapping regions in the genome. Positive

detection of a bacterium, fungus, or parasite required an RPM ratio ≥ 10, where RPM ratio = RPM sample/RPM NTC, or RPM ratio = RPM sample if RPM NTC = 0. The results of mNGS were considered positive when they were consistent with the pathogenicity of positive microorganisms, clinical characteristics, and therapeutic efficacy.

Statistical Analysis

All statistical analyses were performed using SPSS software version 26 (IBM Corporation) and GraphPad Prism 10. Descriptive statistics were used to summarize continuous variables as medians with interquartile ranges (IQRs) for non-normally distributed data and as means with standard deviations (SDs) for normally distributed data. Categorical variables are expressed as frequencies and percentages. The comparison of continuous variables between groups was conducted using the Mann–Whitney *U*-test or Student's *t*-test, depending on the normality of the data. For categorical variables, differences between groups were assessed using the chi-square test or Fisher's exact test, as appropriate. The Kolmogorov–Smirnov test was used to assess the normality of continuous variables. A P-value < 0.05 was considered statistically significant.

Results

Patient Characteristics and Laboratory Findings

This study collected data from 445 patients at the First Affiliated Hospital of Chongqing Medical University, with 367 patients included in the analysis and 334 patients diagnosed with LRTIs. The enrolled patients came from 12 departments, with the majority (78.47%) from the Department of Pulmonology. The median age of the patients was 63 years, and 244 (66.49%) patients were male. A total of 81 (22.07%) patients were admitted to the intensive care unit (ICU), and 136 underwent endotracheal intubation. Among the patients, 76.80% had underlying conditions, with hypertension, diabetes, and chronic obstructive pulmonary disease (COPD) being the most common. The most frequent clinical symptoms were cough, sputum production, and fever. On chest imaging, most patients (91.83%) exhibited multiple lesions (Table 1).

Comprehensive Detection of Pathogenic Microorganisms Using mNGS

First, we analyzed the pathogen spectrum of the enrolled patients using mNGS. DNA sequencing was performed on the BALF samples from all enrolled patients, and RNA sequencing was also conducted on the samples from 169 patients. Using mNGS, pathogens were identified in 316 patients, comprising 93 distinct microorganisms, compared to 38

Table 1 Demographic and Clinical Data of the Enrolled Patients

Characteristics	Patients, n (%)
Age (years)	63 (15–97)
Mechanical ventilation	136 (37.06)
Department	
Department of Pulmonology,	288 (78.47)
Department of Critical Care Medicine	43 (11.72)
Department of Infectious Diseases	16 (4.36)
Patient condition	
LRTIs	334 (91.01)
Severe pneumonia	109 (29.70)
Chronic respiratory disease	94 (25.61)

(*Continued*)

Table 1 (Continued).

Characteristics	Patients, n (%)
Sex	
Male	244 (66.49)
Female	123 (33.51)
Comorbidities	
Hypertension	107 (29.16)
Diabetes	75 (20.44)
COPD	54 (14.71)
Pulmonary malignant tumor	32 (8.72)
Coronary disease	30 (8.17)
Clinical manifestation	
Cough	260 (70.84)
Expectoration	191 (52.04)
Fever	136 (37.06)
Dyspnea	92 (25.07)
Asthma	85 (23.16)
Laboratory parameters (median, range)	
WBC (10^9/L)	8.73 (0.64–52.49)
Percentage of neutrophils (%)	81.2 (6.52–98.3)
ALT (U/L)	26 (6–183)
AST (U/L)	31 (8–523)
CRP (mg/L)	66.8 (2–385)
PCT (ng/mL)	0.14 (0.01–84.08)
Radiographic finding	
Solitary lesion	30 (8.17)
Multiple lesions	337 (91.83)
Outcome	
Death	36 (9.81)

Abbreviations: LRTIs, lower respiratory tract infection diseases; COPD, chronic obstructive pulmonary disease; WBC, white blood cell; ALT, alanine aminotransferase; AST, Aspartate Aminotransferase; CRP, c-reactive protein; PCT, Procalcitonin.

microorganisms detected by RMT. The most frequently detected pathogens by mNGS were human herpesvirus 4 (EBV), followed by *Candida albicans*, human herpesvirus 5 (CMV), *E. faecium*, and the *Mycobacterium tuberculosis complex* (MTBC). In contrast, the most common pathogens identified using RMT were *Candida albicans, K. pneumoniae, P. aeruginosa, A. baumannii*, and *Staphylococcus epidermidis*. Across the combined results of mNGS and RMT, the most commonly identified bacteria were MTBC (n = 49), *E. faecium* (n = 47), *K. pneumoniae* (n = 44), and *P. aeruginosa*

(n = 44). The most frequently detected fungi were *Candida albicans* (n = 81), *Pneumocystis jirovecii* (n = 41), and *Aspergillus fumigatus* (n = 24). The most commonly identified viruses were human herpesviruses, including EBV and CMV. In addition, mNGS detected several rare bacterial pathogens, including *Chlamydia psittaci* (n = 3), *Ureaplasma parvum* (n = 1), and *Ureaplasma urealyticum* (n = 1) (Figure 2).

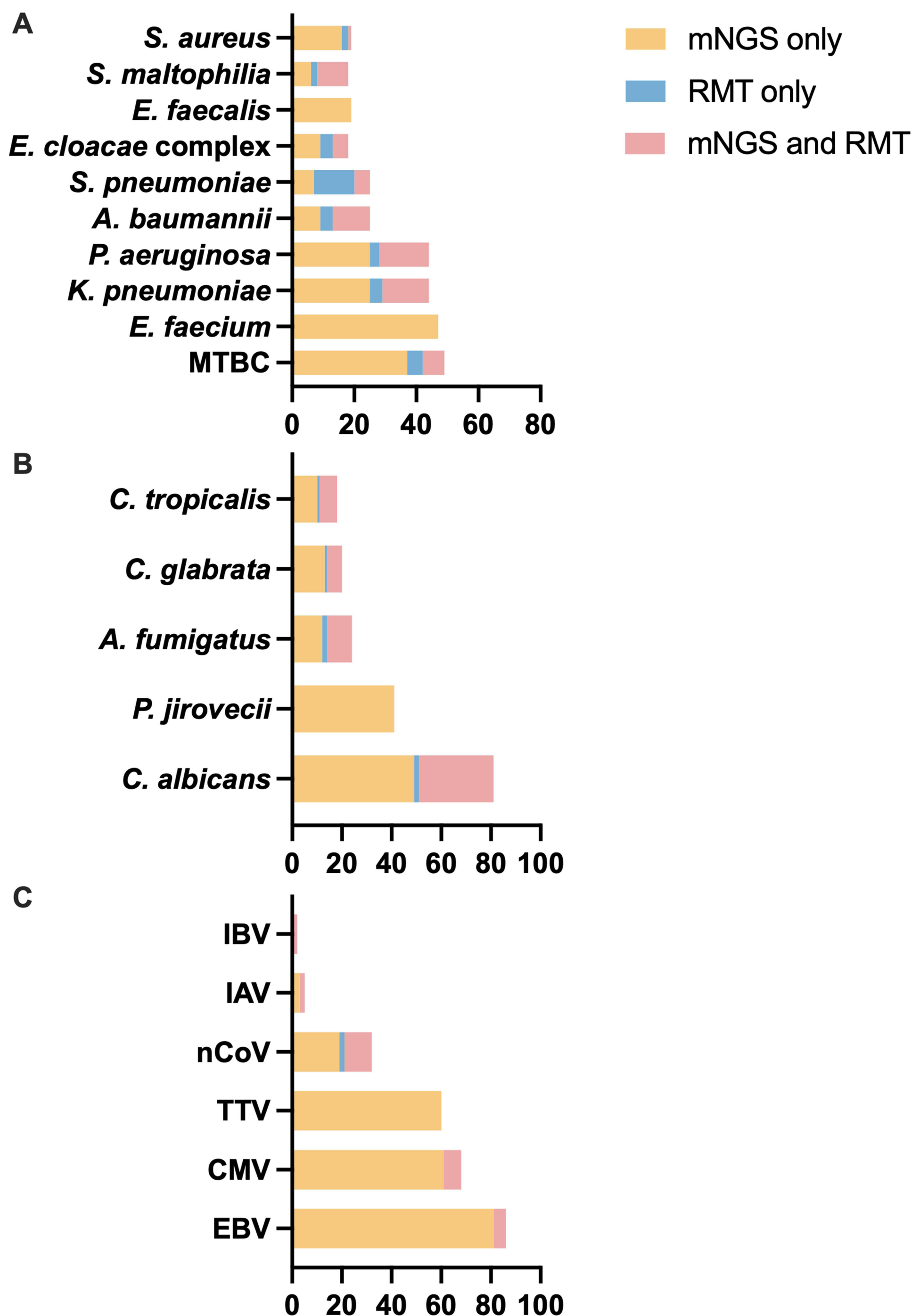

Figure 2 Pathogen spectrum of enrolled patients. (**A**) Top 10 bacterial pathogens. (**B**) Top 5 fungal pathogens. (**C**) Top 6 viral pathogens identified by RMT and mNGS.
Abbreviations: mNGS, metagenomic next-generation sequencing; RMT, routine microbiological testing; *H. influenzae, Haemophilus influenzae; S. aureus, Staphylococcus aureus; S. maltophilia, Stenotrophomonas maltophilia; E. faecalis, Enterococcus faecalis; E. cloacae* complex, *Enterobacter cloacae complex; S. pneumoniae, Streptococcus pneumoniae; A. baumannii, Acinetobacter baumannii; P. aeruginosa, Pseudomonas aeruginosa; K. pneumoniae, Klebsiella pneumoniae; E. faecium, Enterococcus faecium; C. tropicalis, Candida tropicalis; C. glabrata, Candida glabrata; P. jirovecii, Pneumocystis jirovecii; A. fumigatus, Aspergillus fumigatus; C. albicans, Candida albicans*; IBV, Influenza B virus; IAV, Influenza A virus; nCoV, Novel coronavirus; TTV, Torque teno virus; CMV: Cytomegalovirus; EBV: Epstein-Barr Virus.

The results indicate that mNGS significantly outperforms traditional pathogen detection methods in terms of overall pathogen detection rate. This advantage is further highlighted when comparing the microbial spectra of the two methods. Both mNGS and RMT identified 14 types of bacteria, 5 types of viruses, and 14 types of fungi. In addition, mNGS exclusively detected 19 types of bacteria, 17 types of viruses, and 24 types of fungi, while RMT exclusively identified one type of bacterium and one type of fungus (Figure 3). Nearly all bacteria and fungi detected by RMT were also identified by mNGS.

Among patients diagnosed with LRTIs, 110 cases involved single infections: 63 cases of bacterial infection only, 19 cases of fungal infection only, 28 cases of viral infection only. Mixed infections were observed in 206 cases, including 70

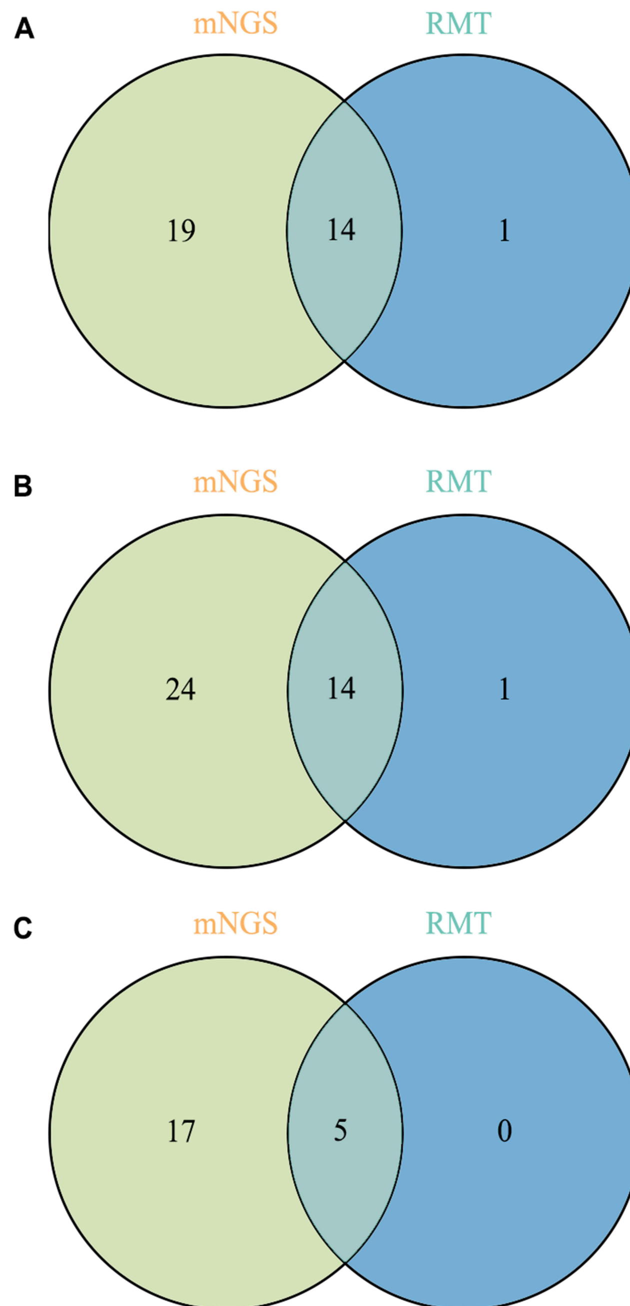

Figure 3 Venn diagram of pathogen detection by mNGS and RMT. The vertical diagram shows the number of bacteria, fungi, and viruses detected by mNGS and RMT, as well as the number of species detected by both methods. (**A**) Bacteria detected by mNGS and RMT. (**B**) Fungi detected by mNGS and RMT. (**C**) Viruses detected by mNGS and RMT.

Abbreviations: mNGS, metagenomic next-generation sequencing; RMT, routine microbiological testing.

cases with concurrent bacterial, fungal, and viral infections; 59 cases with bacterial and viral co-infections; and 41 cases with bacterial and fungal co-infections (Figure 4).

For cases with multiple organisms detected by mNGS, etiologic relevance was determined by clinicians based on relative read abundance, genome coverage, host factors, radiologic findings, and response to targeted therapy. Pathogens considered colonizers were not interpreted as causative agents unless supported by clinical evidence.

Comparison of the Concordance of Pathogen Detection Between mNGS and RMT

To evaluate the consistency between mNGS and different RMT methods, we compared their detection rates. Of the 286 patients with a final diagnosis of LRTI and concurrent BALF bacterial and fungal cultures, 234 remained after excluding pathogenic organisms that could not be cultured in the laboratory. Of these, 37 were negative for both mNGS and BALF cultures, 77 were positive for both, 118 were positive only for mNGS, and 2 were positive only for culture. The culture-positive cases detected *Exophiala dermatitidis* and *Aspergillus fumigatus*, which were probably environmental contaminants. Among the 77 cases positive for both, 18 cases showed complete concordance, and 54 cases showed partial concordance, with 53 cases having more pathogens detected by mNGS compared to culture and only one case showing more pathogens detected by culture. The remaining five cases were discordant (Figure 5). Among the 334 patients diagnosed with LRTIs, the bacterial detection rate of mNGS was 56.58% (189/334), which was significantly higher than the bacterial detection rate of culture at 17.37% (58/334) ($P < 0.0001$). Among the cases subjected to fungal culture, the

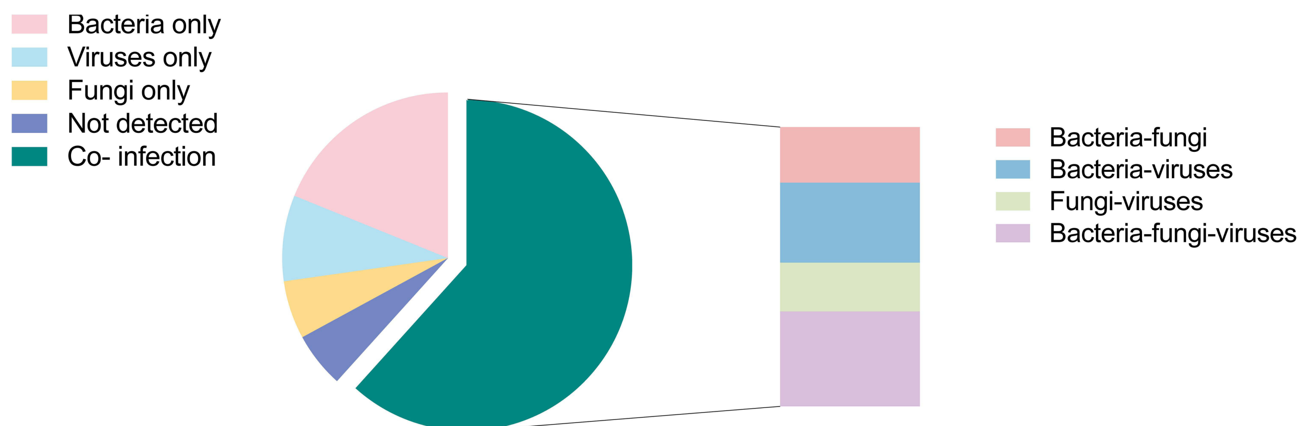

Figure 4 Mixed infections detected by mNGS. The pie chart shows the detection of bacterial, fungal, and viral infections by mNGS alone, whereas the vertical slices chart shows the cases of mixed infections.
Abbreviation: mNGS, metagenomic next-generation sequencing.

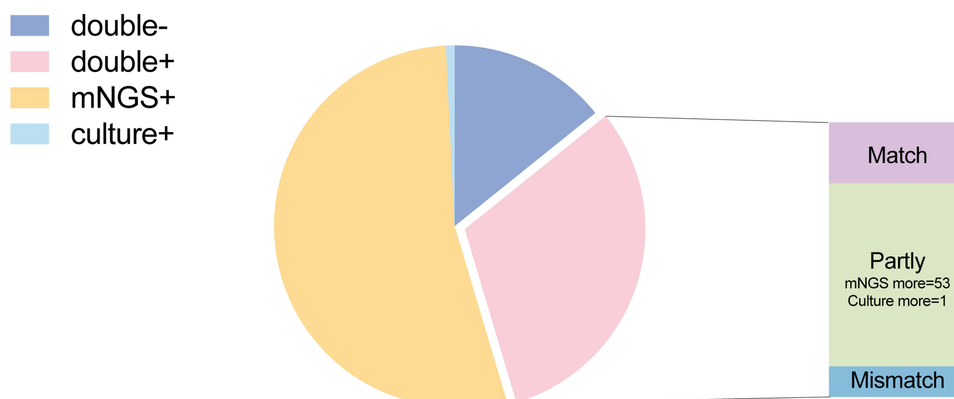

Figure 5 Consistency of mNGS results with culture method results. The pie chart shows the bacterial and fungal infections detected by mNGS and culture, whereas the vertical slices chart shows the specific cases in which both mNGS and culture were positive.
Abbreviation: mNGS, metagenomic next-generation sequencing.

overall fungal detection rate by mNGS was 49.65% (142/286), which was significantly higher than the fungal detection rate by culture at 16.78% (48/286) ($P < 0.0001$). For these cases, the positive rates of Candida and filamentous fungi detected by mNGS were 30.77% (88/286) and 11.89% (34/286), respectively, compared to 10.49% (30/286) and 5.94% (17/286) detected by culture, respectively ($P < 0.05$). Among the 334 patients ultimately diagnosed with LRTIs, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of mNGS were 93.41%, 66.67%, 96.89%, and 50%, respectively. The corresponding values for culture were 27.84%, 96.97%, 98.94%, and 11.72%, respectively.

Furthermore, 175 patients underwent rapid PCR-based infection tests that could simultaneously detect 24 pathogens. Among these, 73 cases were positive for both mNGS and PCR, 61 were negative for both, 35 were positive only for mNGS, and 6 were positive only for PCR. Among the 73 double-positive cases, 32 were completely concordant, while 41 showed partial concordance. Overall, the detection rate of mNGS for these 24 pathogens was 65.14% (114/175), which was significantly higher than the detection rate of PCR at 45.14% (79/175) ($P < 0.05$). In the identification of viruses, 51 patients were tested for EBV and CMV using PCR based on their clinical conditions. EBV was detected in 14 patients using mNGS and in five patients using PCR, all of which were also identified using mNGS. For CMV detection, mNGS identified 16 cases, and PCR identified seven cases; and all seven cases were also detected by mNGS, although there was no statistical significance between the two methods. In addition, 77 patients underwent RNA sequencing and PCR testing for influenza A and B using throat swabs. mNGS detected five cases of influenza A compared to three detected by PCR, and two cases of influenza B compared to two detected by PCR, with mNGS covering all PCR-positive cases (Figure 6).

Among the 94 cases diagnosed with LRTIs that were tested using the tuberculosis Xpert assay, mNGS identified 10 positive cases, whereas Xpert detected 12 positive cases. In addition, 99 patients underwent CrAg testing, with eight cases positive for CrAg, while mNGS identified *Cryptococcus neoformans* in six cases (Figure 6). The missed detections by mNGS were likely due to the low abundance of the pathogens.

Comparative Analysis of the Clinical Characteristics and Pathogen Profiles of Severe and Non-Severe Pneumonia

To clarify the clinical characteristics and diagnostic value of mNGS in different disease severities, we further compared the results between patients with non-severe and severe pneumonia. There were no significant differences between the severe and non-severe pneumonia groups in terms of underlying conditions such as COPD, allergic asthma, bronchiectasis, diabetes, or hypertension. However, significant differences were observed in age, sex, coronary artery disease, white blood cell counts, neutrophil percentages, lymphocyte percentages, C-reactive protein, procalcitonin, and mortality (Table 2).

These results suggest differences in clinical features between the two groups. Therefore, we compared the pathogen profiles between the two groups to determine if the disease severity correlated with the distribution pattern of pathogens (Figure 7A). Across both groups, 16 bacterial species were detected, with nine species uniquely identified in the severe pneumonia group and 16 species uniquely identified in the non-severe pneumonia group (Figure 7B). *Enterococcus faecium* had the highest detection rate in severe pneumonia, whereas *P. aeruginosa* had the highest detection rate in non-severe pneumonia. The detection rates of *E. faecium* ($P < 0.0001$), *A. baumannii* ($P < 0.01$), *Mycobacterium abscessus* ($P < 0.0001$), *Stenotrophomonas maltophilia* ($P < 0.05$), and *E. coli* ($P < 0.05$) were statistically significant between the two groups. For fungal detection, nine species were identified in both groups, with 6 species unique to the severe pneumonia group and 10 species unique to the non-severe pneumonia group. The detection rates of *Pneumocystis jirovecii, Candida tropicalis*, and *Candida parapsilosis* were statistically significant ($P < 0.05$). Regarding DNA viruses, seven species were identified in both groups, with two species uniquely detected in the severe pneumonia group and two species uniquely detected in the non-severe pneumonia group. The detection rate of human herpesvirus 1 showed statistical significance between the two groups ($P < 0.01$).

In the severe pneumonia group, 78.90% (86/109) of patients had mixed infections, with the most common type being bacterial-fungal-viral co-infections. In the non-severe pneumonia group, 53.78% (121/225) of the patients had mixed infections ($P < 0.001$) (Figure 7C).

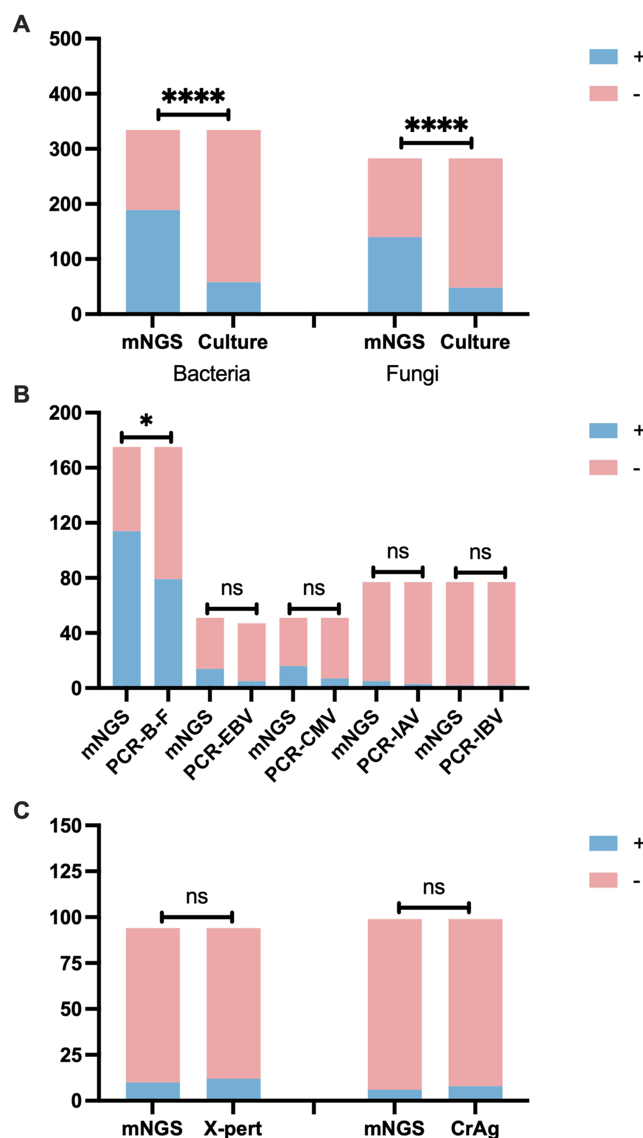

Figure 6 Comparison of positivity rates between mNGS and RMT. (**A**) Detection rates of bacteria and fungi by mNGS and culture. The number of positive samples (y-axis) for pairwise mNGS and culture testing is plotted against the number of bacteria and fungi (x-axis). (**B**) The positive detection rates of bacteria, fungi, EBV, CMV, and influenza A/B by mNGS and PCR. (**C**) The positive detection rates of *Mycobacterium tuberculosis* complex by mNGS and Xpert and of cryptococcal capsular antigen by mNGS and CrAg testing. *P < 0.05; ****P < 0.0001.

Abbreviations: mNGS, metagenomic next-generation sequencing; RMT, routine microbiological testing; Xpert, xpert Mycobacterium tuberculosis /rifampicin resistance; CrAg, Cryptococcus capsular polysaccharide antigen; PCR, Polymerase Chain Reaction; PCR-B-F, PCR-based bacterial and fungal detection; CMV, Cytomegalovirus; EBV, Epstein-Barr Virus; IAV, Influenza A Virus; IBV, Infectious Bronchitis Virus; ns, not significant.

Consistency Evaluation of mNGS Resistance Genes with Antimicrobial Susceptibility Test Results

In addition to identifying the pathogenic microorganisms of LRTIs, mNGS can simultaneously detect resistance genes against various antimicrobial agents, such as macrolides, carbapenems, and β-lactams, providing an important reference for clinical precision antimicrobial agent use and optimization of antimicrobial strategies. In our results, no specimens tested positive for vancomycin-resistant *Enterococcus* (VRE) or methicillin-resistant *Staphylococcus aureus* (MRSA); therefore, a comparison of these resistance genes could not be conducted. To assess the accuracy of mNGS in predicting resistance genes, 49 specimens that were positive in both mNGS and culture were selected for comparative analysis (in four of the cases, two pathogenic microorganisms were detected in the specimens), and all specimens underwent antimicrobial susceptibility testing (AST) (Table 3 and Figure 8). The results showed that in 24 specimens, no resistance genes were detected by mNGS, and the AST

Table 2 Comparison of Clinical Characteristics Between Severe and Non-Severe Pneumonia Groups

Variable	Severe Pneumonia (n = 109)	Non-Severe Pneumonia (n = 225)	*P*
Age, median (IQR), years	68	61	0.0016
Sex, n (%)			
Male	84	141	0.0085
Female	25	84	
Comorbidities, n (%)			
Hypertension	35 (32.7)	62 (27.8)	0.3597
COPD	18 (16.8)	32 (14.3)	0.5576
Diabetes	25 (23.4)	45 (20.2)	0.5076
Coronary heart disease	17 (15.9)	12 (5.4)	0.0016
Laboratory parameters			
WBC (10^9/L)	10.63	8.1	0.0004
Percentage neutrophils (%)	88.7	75.9	< 0.0001
Percentage of lymphocytes (%)	6.5	13.7	< 0.0001
ALT (U/L)	29	25	0.0033
AST (U/L)	42	28	< 0.0001
CRP (mg/L)	98.6	35.65	< 0.0001
PCT (ng/mL)	0.32	0.08	< 0.0001
Outcome, Mortality, n (%)	26 (24.2)	10 (4.4)	< 0.0001

Abbreviations: COPD, chronic obstructive pulmonary disease; WBC, white blood cell; ALT, alanine aminotransferase; AST, Aspartate Aminotransferase; CRP, c-reactive protein; PCT, Procalcitonin.

results indicated sensitivity to all tested antimicrobials except intrinsic resistant antimicrobials, demonstrating concordance between the mNGS predictions and the AST results. For the remaining specimens, *A. baumannii* were detected in 11 specimens, of which three specimens were found to be $bla_{OXA\text{-}23}$ positive, with AST confirming that all three specimens were cultured with carbapenem-resistant *A. baumannii* (CRABA); seven specimens were found to be $bla_{\mathrm{OXA\text{-}23}}$ and bla_{TEM} positive, with AST confirming that all of the seven specimens were cultured with CRABA and showed resistance to third-generation cephalosporins; and one specimen was found to be bla_{NDM} and $bla_{\mathrm{OXA\text{-}23}}$ positive, and AST revealed the strain to be CRABA. These findings showed high concordance between the mNGS-detected resistance genes and the AST results. *Klebsiella pneumoniae* was detected in eight specimens, two of which were found to be positive for bla_{TEM}, bla_{SHV}, $bla_{\mathrm{CTX\text{-}M}}$, and bla_{KPC}. AST confirmed that these two specimens were cultured with carbapenem-resistant *K. pneumoniae* (CRKP) and resistant to third-generation cephalosporins. Three specimens were found to be positive for bla_{SHV} alone; one specimen was found to be positive for $bla_{\mathrm{CTX\text{-}M}}$, bla_{SHV}, and bla_{TEM}; one specimen was found to be positive for bla_{SHV} and bla_{TEM}; and one specimen was found to be positive for bla_{TEM} alone. AST confirmed that all specimens were cultured with extended-spectrum beta-lactamase (ESBL) production. These results demonstrated that the mNGS predictions were generally consistent with the AST results. *Pseudomonas aeruginosa* was detected in five specimens, one of which was found to be positive for bla_{TEM}, while AST was cultured with carbapenem-resistant *P. aeruginosa* (CRPAE). mNGS did not detect resistance genes in the remaining four specimens, but AST identified them as CRPAE. This suggests that mNGS may miss resistance mechanisms such as outer membrane protein

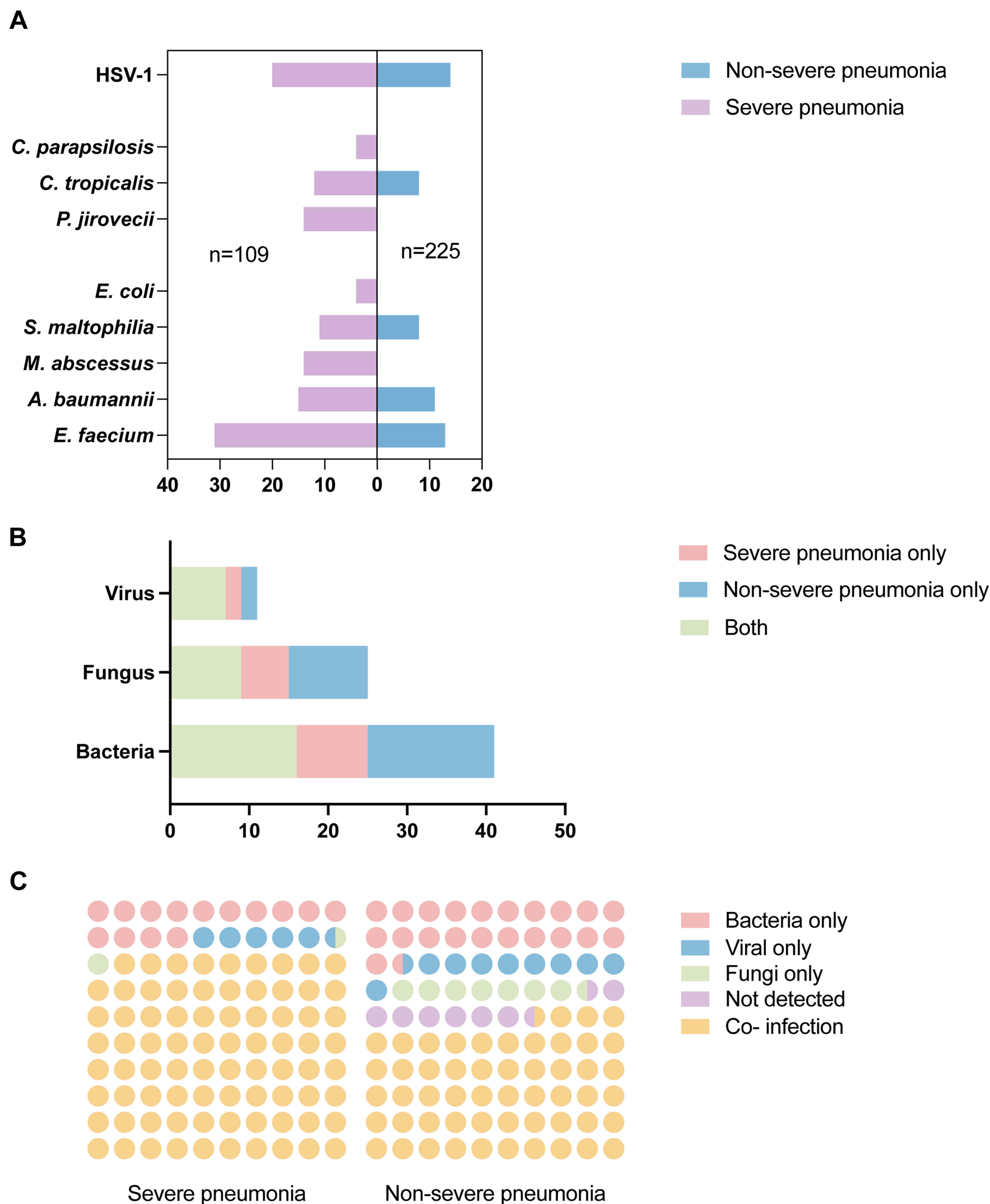

Figure 7 Comparison of pathogen detection rates and infection conditions between the severe and non-severe pneumonia groups. (**A**) Comparison of pathogens between the severe and non-severe pneumonia groups, with statistical significance for the listed pathogens in both groups ($P < 0.05$). (**B**) The number of bacteria, fungi, and viruses detected in the severe and non-severe pneumonia groups. (**C**) Infection profiles detected by mNGS in the severe and non-severe pneumonia groups.

Abbreviations: mNGS, metagenomic next-generation sequencing; *E. faecium, Enterococcus faecium; A. baumannii*, Acinetobacter baumannii; M. abscessus, Mycobacterium abscessus; *S. maltophilia, Stenotrophomonas maltophilia; E. coli, Escherichia coli; P. jiroveci, Pneumocystis jirovecii; C. tropicalis, Candida tropicalis; C. parapsilosis, Candida parapsilosis.*

https://doi.org/10.2147/IDR.S572028

Table 3 Comparison of Antimicrobial Resistance Genes Detected Using mNGS and Antimicrobial Susceptibility Testing Results From Cultures

Sample ID	Pathogens	Antimicrobial Resistance	
		mNGS Detected Resistance Gene(s)	**AST Phenotype–Resistant Antimicrobials**
P2	*Klebsiella pneumoniae*	$bla_{CTX\text{-}M}$ bla_{KPC} bla_{SHV} bla_{TEM}	CRE
P4	*K. pneumoniae*	$bla_{CTX\text{-}M}$ bla_{KPC} bla_{SHV} bla_{TEM}	CRKP
P8	*Acinetobacter baumannii*	$bla_{OXA\text{-}23}$ bla_{TEM}	CRABA
P14	*A. baumannii*	$bla_{OXA\text{-}23}$ bla_{TEM}	CRABA
P20	*Enterobacter cloacae complex*	bla_{IMP}	CRE
P23	*K. pneumoniae*	bla_{SHV}	ESBLs
	A. baumannii	$bla_{OXA\text{-}23}$ bla_{TEM}	CRABA
P35	*Pseudomonas aeruginosa*	–	CRPAE
P48	*A. baumannii*	$bla_{OXA\text{-}23}$	CRABA
P78	*Enterobacter cloacae complex*	bla_{NDM}	CRE
	A. baumannii	bla_{NDM} $bla_{OXA\text{-}23}$	CRABA
P81	*A. baumannii*	$bla_{OXA\text{-}23}$ bla_{TEM}	CRABA
P99	*A. baumannii*	$bla_{OXA\text{-}23}$	CRABA
P105	*P. aeruginosa*	–	CRPAE
P107	*A. baumannii*	$bla_{OXA\text{-}23}$ bla_{TEM}	CRABA
P138	*Enterobacter cloacae complex*	bla_{NDM} bla_{TEM}	CRE
P143	*K. pneumoniae*	$bla_{CTX\text{-}M}$ bla_{SHV} bla_{TEM}	ESBLs
P170	*K. pneumoniae*	bla_{SHV}	ESBLs
P195	*A. baumannii*	$bla_{OXA\text{-}23}$ bla_{TEM}	CRABA
P219	*A. baumannii*	$bla_{OXA\text{-}23}$ bla_{TEM}	CRABA
P244	*K. pneumoniae*	bla_{SHV} bla_{TEM}	ESBLs
P269	*K. pneumoniae*	bla_{SHV}	ESBLs
P298	*P. aeruginosa*	bla_{TEM}	CRPAE
	K. pneumoniae	bla_{TEM}	ESBLs
P323	*Streptococcus pneumoniae*	*ErmB*	Erythromycin; Clindamycin
P334	*P. aeruginosa*	–	CRPAE
P343	*Haemophilus influenzae*	bla_{TEM}	Ampicillin; TMP-SMX
P360	*A. baumannii*	$bla_{OXA\text{-}23}$	CRABA
	P. aeruginosa	–	CRPAE

Abbreviations: mNGS, metagenomic next-generation sequencing; CRE, Carbapenem-resistant Enterobacterales; AST, Antimicrobial Susceptibility Testing; CRKP, Carbapenem-resistant *Klebsiella pneumoniae*; CRABA, Carbapenem-resistant *Acinetobacter baumannii*; ESBLs, Extended-Spectrum β-Lactamases; CRPAE, Carbapenem-resistant *Pseudomonas aeruginosa*; TMP-SMX, Trimethoprim - Sulfamethoxazole.

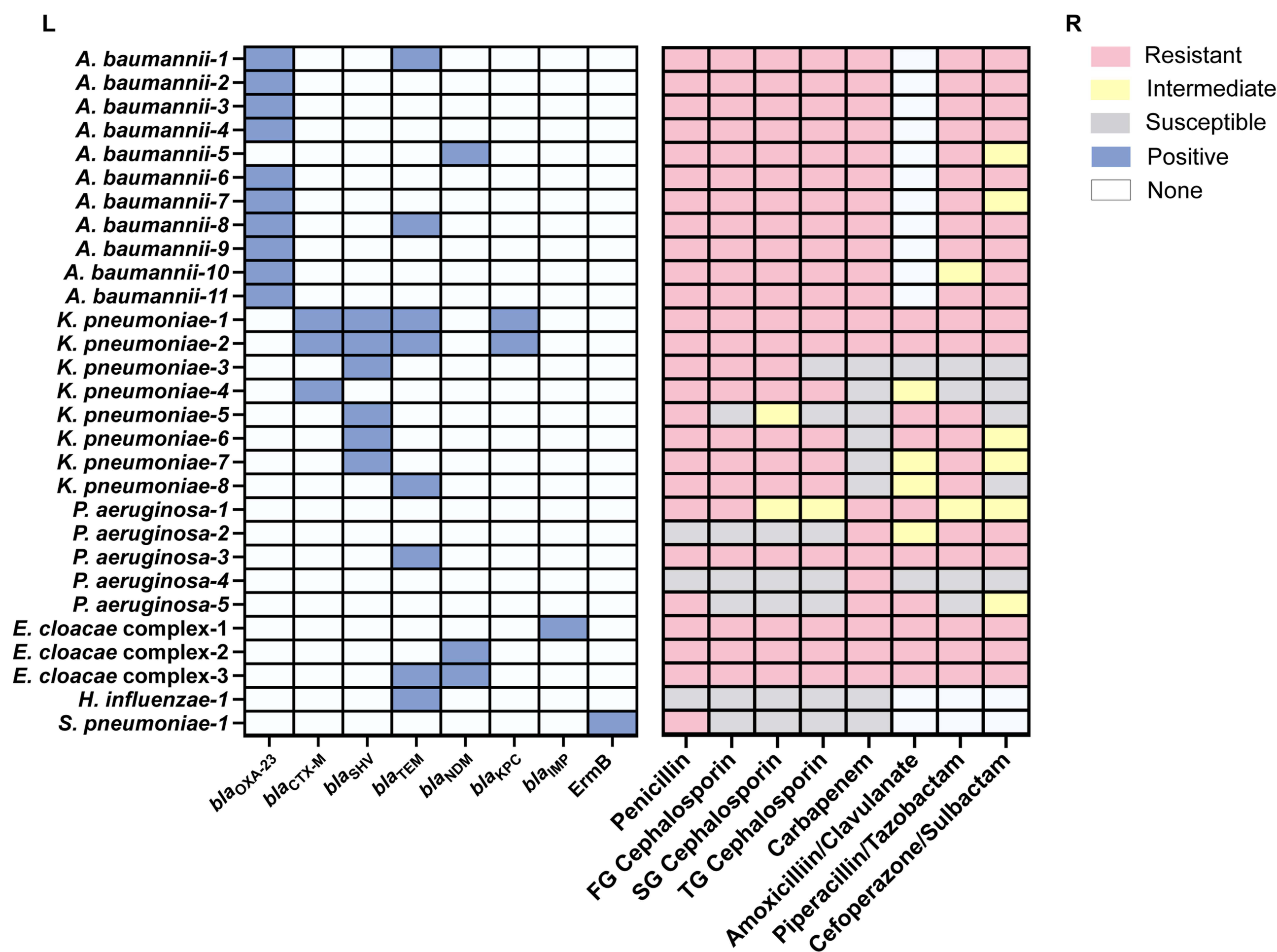

Figure 8 Comparison of bacterial resistance detected using both mNGS and culture. Resistance genes identified using mNGS (left) and AST (right).
Abbreviations: mNGS, metagenomic next-generation sequencing; *A. baumannii, Acinetobacter baumannii; K. pneumoniae, Klebsiella pneumoniae; P. aeruginosa, Pseudomonas aeruginosa; E. cloacae complex, Enterobacter cloacae complex; H. influenzae, Haemophilus influenzae; S. pneumoniae, Streptococcus pneumoniae*; AST, Antimicrobial Susceptibility Testing; FG, First generation; SG, Second generation; TG, Third generation.

mutations and efflux pump overexpression. The *Enterobacter cloacae complex* was detected in three specimens, one of which was found to be positive for bla_{IMP}, one was positive for bla_{NDM}, and one was positive for both bla_{IMP} and bla_{TEM}, with AST confirming that all three specimens were cultured with carbapenem-resistant *Enterobacteriaceae* (CRE), demonstrating consistency between the mNGS findings and AST results. *Streptococcus pneumoniae* was detected in one specimen, which was found to be positive for *ermB*, with AST confirming that the specimen was cultured with resistance to erythromycin and clindamycin, showing concordance between the mNGS predictions and AST results.

In summary, in most cases, the antimicrobial resistance genes detected by mNGS were consistent with the AST results, particularly in *A. baumannii, K. pneumoniae*, and the *Enterobacter cloacae complex*. However, in *P. aeruginosa*, there were cases in which mNGS did not detect resistance genes, yet AST showed resistance, suggesting that mNGS may have limitations in detecting certain resistance mechanisms or that resistance may be mediated by non-genotypic mechanisms such as reduced outer membrane permeability or overexpression of efflux pumps. These results highlight the importance of combining genotypic and phenotypic testing to comprehensively assess the antimicrobial resistance of pathogens.

Impact of mNGS on Clinical Antimicrobial Therapy Adjustments

To further assess the practical value of mNGS results in guiding clinical antimicrobial adjustments, patients were categorized into an antimicrobial-exposure group and a non-exposure group based on whether empirical antimicrobial treatment was administered prior to specimen submission; this allowed us to analyze the adjustments to antimicrobial regimens guided by the pathogen spectrum identified through mNGS (Figure 9). In the antimicrobial-exposed group (n = 334), the findings indicated

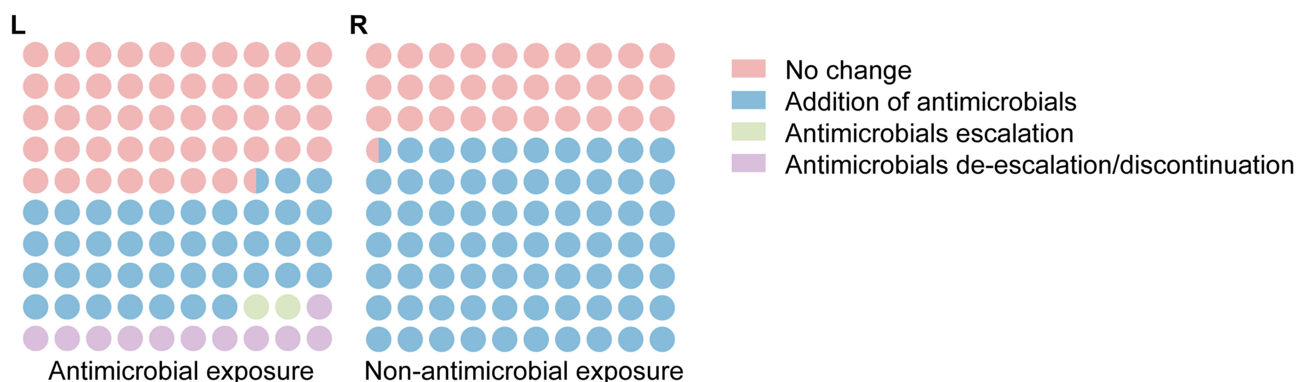

Figure 9 Adjustments to antimicrobials based on mNGS results in the antimicrobial-exposed and non- antimicrobial-exposed groups. The left panel shows the condition of the antimicrobial -exposed group, and the right panel shows the condition of the non- antimicrobial-exposed group.
Abbreviation: mNGS, metagenomic next-generation sequencing.

that mNGS positively influenced treatment decisions in the majority of cases, including confirming the initial antimicrobial targeted treatment (n = 158), guiding antimicrobial escalation (n = 8), and advising on the addition of antimicrobials (n = 127), including 89 cases where antifungal agents were added due to fungal detection by mNGS. mNGS also guided the de-escalation or discontinuation of antimicrobials in 36 cases, including 17 cases in which antimicrobials were stopped because of negative mNGS results. mNGS had no impact on antimicrobial guidance in five cases where clinicians added medications based on RMT or other factors (the specific information is shown in Table 4). In the non-exposed group (n = 33), mNGS had a positive impact in most patients: 23 patients received additional therapy based on the mNGS results, whereas nine patients did not receive antimicrobials due to negative mNGS findings. In one case, human herpesvirus type 7 was detected by mNGS, but no clinical treatment was administered. Overall, the study demonstrated that among 194 patients whose treatment was adjusted based on mNGS results, 87.11% (179 cases) showed symptom improvement and were successfully discharged, highlighting the potential value of mNGS in optimizing clinical therapy and improving patient outcomes.

On this basis, we further investigated the impact of prior antimicrobial exposure on the positive detection rates of mNGS and traditional cultures. Among the 321 patients who had received empirical antibacterial treatment, the bacterial detection rate of mNGS was 67.60% (217/321), which was significantly higher than that of culture at 55.14% (177/321) (P < 0.05). In 72 cases with empirical antifungal therapy and subsequent fungal culture, the fungal detection rate of mNGS was 66.67% (48/72), which was significantly higher than that of culture at 22.22% (16/72) (P < 0.05). However, in 33 patients who had not received empirical antimicrobial treatment, there was no significant difference in the positive detection rates between the two methods (P = 0.084). These findings suggest that compared to RMT, the detection efficiency of mNGS is not affected by prior antimicrobial exposure.

Table 4 Five Specific Cases in Which mNGS Did Not Influence the Antimicrobial Treatment Decision

Sample ID	mNGS Results	RMT Results (Sample Types)	Clinical Diagnosis	Additional Antimicrobial Therapy	Notes
P66	Not detected	–	*Actinomycosis*	Penicillin	Diagnosis based on clinical history and presentation
P120	Not detected	Xpert MTB/RIF positive (BALF)	*M. tuberculosis*	Anti-tuberculosis therapy	
P168	Not detected	CrAg positive (serum)	*Cryptococcosis*	Fluconazole	
P233	*S. constellatus*	Culture: *P. aeruginosa* (BALF)	*P. aeruginosa*	Piperacillin-Tazobactam	
P304	Not detected	CrAg positive (serum)	*Cryptococcosis*	Fluconazole	

Abbreviations: mNGS, Metagenomic next-generation sequencing; RMT, routine microbiological testing; *S. epidermidis, Staphylococcus epidermidis; M. tuberculosis, Mycobacterium tuberculosis*; CrAg, Cryptococcal antigen; *P. aeruginosa, Pseudomonas aeruginosa*.

Table 5 Information on Patients Infected with Hypervirulent *Klebsiella Pneumoniae*

Patient	Department	Underlying Conditions	Resistance Gene	Treatment Regimen	Outcome
hvKP-1	Department of Pulmonology	Hypertension Renal insufficiency Fatty liver Diabetes	bla_{SHV}	Cefoperazone-Sulbactam	Improvement
hvKP-2	ICU	Hypertension Diabetes	–	Meropenem	Improvement
hvKP-3	ICU	Viral hepatitis	bla_{SHV}	Meropenem	Improvement

Abbreviations: hvKP, Hypervirulent Klebsiella pneumoniae; ICU, Intensive Care Unit.

Enhanced Identification and Characterization of Hypervirulent Klebsiella pneumoniae Using mNGS

Virulence genes, particularly hypervirulent genes of *K. pneumoniae*, detected by mNGS provide vital indications for infection treatment. To further elucidate the advantages of mNGS in detecting and typing *K. pneumoniae*, we compared the results of mNGS and traditional culture methods. mNGS detected 43 cases of *K. pneumoniae*, whereas traditional culture identified only 14 cases ($P < 0.001$), indicating that mNGS is more sensitive in detecting *K. pneumoniae*. Among the 43 mNGS-positive specimens, three were identified as hypervirulent *K. pneumoniae* (hvKP), and all detected the *iutA* and *rmpA* genes. All three cases were admitted to the Department of Pulmonology, with two requiring ICU admission and one diagnosed with severe pneumonia. Of the three cases, two had diabetes and one had chronic hepatitis B virus infection with chronic liver disease. Patients with diabetes subsequently developed metastatic lesions, including in the lung and liver, and meningitis. In the resistance gene analysis, two patients carried the bla_{SHV} resistance gene. For treatment, two patients received carbapenem antimicrobials, while one received a combination of broad-spectrum penicillin and beta-lactamase inhibitors. Detailed case information is presented in Table 5.

Discussion

With the advancement of high-throughput sequencing technologies, second-generation metagenomic sequencing has seen a significant increase in its application for detecting pathogens in LRTIs. mNGS does not require assumptions about the type of pathogen, thereby enabling comprehensive screening for all known and unknown microorganisms in a single test.[13,14] This capability allows for better treatment of patients with mixed pathogen infections.

In this study, we found that mNGS not only rapidly and comprehensively identified potential pathogens but was also unaffected by antimicrobial exposure and demonstrated high accuracy in predicting resistance genes.[15] mNGS demonstrated higher sensitivity in detecting pathogens than traditional methods, particularly for specific pathogens.[16] In our study, *Chlamydia psittaci, Ureaplasma parvum*, and *Ureaplasma urealyticum* were exclusively detected by mNGS, highlighting its advantage in detecting rare pathogens. Furthermore, in our study, mNGS detected bacteria and fungi, and the overall bacterial and fungal detection rates were 56.58%, 49.65%, and 65.14%, respectively, all of which were higher than those of bacterial culture, fungal culture, and PCR methods. These findings agree with previous research results.[16–18] However, in detecting *Mycobacterium tuberculosis* complex (MTBC), the detection rate of mNGS was 10.64%, which was lower than that of Xpert (12.77%), with no statistical significance. Liu et al[19] also reported that the sensitivity of mNGS for detecting MTBC was lower than that of Xpert. This discrepancy can be attributed to fundamental differences in their methodologies. Unlike Xpert, mNGS directly sequences nucleic acids without prior amplification. The relatively low abundance of *Mycobacterium tuberculosis* (*M. tuberculosis*) DNA in clinical specimens may reduce the detection sensitivity of mNGS. In contrast, Xpert is a real-time PCR-based method that uses highly specific primers designed to detect *M. tuberculosis* and rifampicin gene mutations. By targeting specific genomic regions for amplification and detection, Xpert maximizes pathogen preservation and detection, resulting in higher sensitivity and specificity compared to mNGS.

mNGS includes DNA and RNA sequencing technologies, facilitating comprehensive genomic and transcriptomic analyses of microbial communities. DNA sequencing is primarily used to detect bacteria, DNA viruses, and other pathogens in samples, whereas RNA sequencing has a distinct advantage in detecting RNA viruses (eg, influenza virus, coronaviruses). During the early outbreak of COVID-19, researchers in Wuhan identified the novel coronavirus through RNA sequencing using NGS.[20] Therefore, dual sequencing provides comprehensive coverage of potential pathogen types, enhancing detection accuracy, particularly in cases of complex infections[21] and in patients with suspected respiratory viral infections. In our study, 169 patients underwent extra RNA sequencing, revealing RNA viruses in 73 cases, highlighting the remarkable capability of mNGS in identifying RNA pathogens. In addition, RNA viruses were the sole pathogens detected in five cases, highlighting the critical role of RNA sequencing in diagnosing respiratory infections. As this study focused on patients with suspected LRTIs, in which most respiratory pathogens are RNA viruses, robust RNA sequencing can minimize the likelihood of false-negative results. This dual sequencing strategy significantly improved the overall pathogen detection rate and helped identify pathogens that might be missed by traditional methods, thereby providing more comprehensive microbial data to guide clinical targeted therapies. Furthermore, RNA sequencing can detect the transcriptional activity of pathogens, thereby aiding in distinguishing active infections from non-active infections. DNA fragments detected by mNGS may originate from dead pathogens, whereas RNA typically represents actively replicating pathogens, which is crucial for optimizing treatment strategies.[21]

Literature indicates that the pathogen types in patients with severe pneumonia are more complex, often presenting as mixed infections, and are associated with antimicrobial resistance.[7,22] Therefore, the advantages of mNGS are particularly evident in these patients. Currently, the treatment of severe pneumonia primarily involves anti-infection therapies and the management of complications. The timely detection of pathogens in patients with severe pneumonia can significantly improve treatment strategies and prognosis. In this study, we found that pathogens in the severe pneumonia group were more diverse and predominantly involved mixed infections, whereas single infections were more common in the non-severe pneumonia group. Furthermore, this study revealed that mNGS demonstrated a significantly higher pathogen detection rate in the severe pneumonia group compared to traditional methods, particularly for mixed infections and the identification of specific pathogens. In our study, *E. faecium* was the most frequently detected pathogen in severe pneumonia patients (45%), with statistical significance. This suggests that this pathogen may have potential clinical significance in such patients. Although *E. faecium* is commonly colonized in the gut and oral cavity, it can also cause pulmonary infections in immunosuppressed patients, patients with prolonged antimicrobials exposure, and those with ventilator-associated pneumonia.[23] Furthermore, as a high-throughput detection tool, mNGS demonstrates high sensitivity for detecting *E. faecium*. However, it also carries the possibility of misidentifying colonizing bacteria as pathogens. Therefore, clinical interpretation should integrate the patient's medical history, imaging findings, and treatment response to avoid excessive or unnecessary antibiotic therapy. Hospitals are high-risk environments for *E. faecium* transmission, which, despite disinfection efforts, can persist in the environment and recontaminate wards,[24] entering the body through invasive procedures, such as intubation, catheterization, or central venous access, or directly via environmental contamination. This study conducted a preliminary analysis of the detection of *E. faecium* in combination with clinical data. However, due to the limited sample size, further research is needed to clarify its clinical significance. In addition, pathogens such as *A. baumannii* and *E. coli* were detected more frequently in the severe pneumonia group than in the non-severe pneumonia group. We hypothesized that the use of broader and higher-tier antimicrobials in patients with severe pneumonia may lead to dysbiosis, providing these pathogens with a competitive advantage for survival and proliferation.

In addition to its significant advantages in pathogen detection, mNGS can simultaneously detect resistance genes present in bacteria. In this research, we compared the resistance genes identified by mNGS to the AST results and found high concordance in most cases (48/53, 90.57%). Previous studies have reported an 86.67% concordance between mNGS resistance detection and AST results.[5] This demonstrates that mNGS has excellent reliability and clinical value for resistance evaluation. mNGS can rapidly provide resistance gene information before traditional AST results are available, enabling faster optimization of antimicrobial selection and reducing the risk of antimicrobial overuse associated with empirical therapy. However, some discrepancies were observed in our results, where mNGS failed to detect resistance genes or showed inconsistency with AST. These differences may stem from various factors, including a low pathogen sequence abundance affecting the detection of resistance genes from a technical perspective, or the presence of rare or

novel resistance genes or resistance mechanisms that are incompletely understood from a biological mechanism standpoint.[15,25–27] Sample contamination, improper handling, DNA degradation, or inhibitors may also contribute to these discrepancies.[28] These limitations highlight areas where mNGS can be improved. In this study, six isolates of *P. aeruginosa* showed discordance with AST results, likely due to the complex resistance mechanisms of *P. aeruginosa*, including reduced outer membrane permeability and overexpression of efflux pumps.[29] Therefore, mNGS cannot entirely replace AST. Although AST provides direct in vitro sensitivity of bacteria to antimicrobial agents, mNGS serves as a complementary tool, which is particularly valuable in rapid diagnostics, detecting rare resistance genes, and guiding treatment of complex infections.

In most cases, clinicians empirically administer antimicrobials before obtaining the etiological results. Prior use of these antimicrobials can affect the detection rate of RMT, particularly in culture-based methods. By analyzing the relationship between antimicrobial use and the positive rates of mNGS and culture, we found that among 341 patients receiving empirical antimicrobial therapy, mNGS demonstrated a significantly higher positive detection rate compared to traditional culture methods. This result indicates that antimicrobial use significantly impacts the sensitivity of traditional culture methods but has a minimal effect on mNGS detection efficiency. Previous studies have also explored the performance of mNGS and traditional methods under different clinical medication conditions, consistently demonstrating that mNGS detection rates are less affected by antimicrobials use.[30,31] One probable reason for this is that antimicrobials inhibit or kill live bacteria in the sample, preventing pathogen growth in culture and thereby reducing the positive rate.[9,18,32] However, mNGS can detect the nucleic acid sequences of pathogens, as well as their DNA or RNA fragments.

In our study, *K. pneumoniae* was detected in 43 cases via mNGS, whereas only 14 cases were identified via culture. Among these 43 cases, three were identified as hvKP carrying *iutA* and *rmpA* genes by mNGS. Two of these hvKP cases were simultaneously identified as being positive for resistance genes, whereas the remaining case did not exhibit any detected resistance genes. Although traditionally hypervirulent strains are considered sensitive to most antimicrobials, recent reports have highlighted the emergence of strains that exhibit both hypervirulence and multidrug resistance globally.[33–36] These strains pose significant clinical challenges because of their capacity to cause severe infections and resistance to conventional antimicrobial therapies. The development of resistance may occur through the acquisition of resistance genes, particularly via plasmid-mediated gene transfer, such as those encoding ESBLs or carbapenemases.[33] These resistance genes can be horizontally transferred among bacteria, thereby rendering previously susceptible hypervirulent strains resistant. In the three hvKP cases, one patient had prior sputum cultures indicating CRKP. Upon admission, BALF was collected and analyzed by both mNGS and traditional culture. hvKP was detected only by mNGS, whereas culture yielded negative results. Due to the symptoms of headache, fever, and lethargy, clinicians suspected meningitis and submitted the patient's cerebrospinal fluid sample for mNGS testing, which also detected *K. pneumoniae*. Subsequent computed tomography scan revealed the formation of lung and liver abscesses. These clinical manifestations align with the characteristics of hvKP, which is highly invasive and prone to cause severe diseases, such as meningitis and lung and liver abscesses.[35] hvKP strains carry multiple virulence factors, including capsular polysaccharides, siderophores, and adhesions, enhancing their ability to evade host immunity and invade tissues.[37] The use of mNGS, which can simultaneously detect resistance and virulence genes, provides a more comprehensive understanding of the infection, facilitates timely therapeutic decisions, and improves patient outcomes.

Importantly, final etiological determination in this study relied on comprehensive clinical assessment rather than mNGS results alone. Because mNGS can detect both pathogens and colonizing organisms, its findings must be interpreted in the clinical context to avoid overdiagnosis. For example, although *Candida species* were frequently detected, they were regarded as colonizers unless imaging features, G/GM results, immunological status, or therapeutic response supported a true infection. The relatively high *Candida* detection rate was largely attributable to the characteristics of the study population, as many positive cases occurred in immunocompromised individuals or in patients heavily exposed to broad-spectrum antibiotics—conditions known to predispose to opportunistic fungal disease. In several such cases, elevated fungal biomarkers, consistent radiologic findings, and clinical improvement after antifungal therapy jointly indicated a pathogenic rather than colonizing role.

However, this study has some limitations. First, as a single-center retrospective analysis, patient enrollment may have been influenced by institutional characteristics, introducing potential selection bias. In addition, some patients received antimicrobial therapy before admission, which may have reduced the positivity rate of conventional cultures and thereby

affected comparisons across diagnostic modalities. Second, although we restricted mNGS testing to BALF samples, routine clinical evaluation often involves additional specimen types (eg, blood, swabs, or serum) for routine microbiological testing. While these tests aid clinical decision-making, the diagnostic information from different specimen types is not fully comparable, which may influence interpretation of the findings. Finally, the relatively high cost of mNGS, its complex workflow, and the need for specialized bioinformatics expertise may limit its accessibility across healthcare settings. Overall, these limitations underscore the need for larger, multicenter, prospective studies to further validate the clinical utility of mNGS and to determine optimal strategies for integrating mNGS with routine diagnostics and clinical assessment.

Conclusions

In conclusion, mNGS substantially improves pathogen detection rates in patients with LRTIs, particularly for fastidious, atypical, or mixed infections that are often missed by routine tests. The study also demonstrated that mNGS provides useful information for antimicrobial decision-making, including the identification of potential resistance genes that may help refine treatment strategies. However, these benefits must be interpreted with caution: mNGS results require clinical correlation, phenotypic susceptibility testing remains essential, and factors such as cost, and technical requirements currently limit widespread implementation. Therefore, mNGS should be viewed as a valuable adjunct—rather than a replacement—to standard microbiological diagnostics in the management of lower respiratory tract infections.

Data Sharing Statement

Sequence data that support the findings of this study have been deposited in the National Genomics Data Center (NGDC) (https://ngdc.cncb.ac.cn/) with reference number PRJCA037984.

Ethics Approval and Consent to Participate

The study was approved by the ethical research committee of the First Affiliated Hospital of Chongqing Medical University (Approval No. 2024-206-01) and was conducted in accordance with the Declaration of Helsinki. As the study was based on a retrospective review of anonymous medical records and did not involve patient interaction, informed consents of the patients were waived by the ethical research committee of the First Affiliated Hospital of Chongqing Medical University.

Acknowledgments

We would like to sincerely thank WR, WQ, PS, WH, DY, and BX for conducting the mNGS testing. We would also like to thank SJ, HS, and XX for their contributions in interpreting the mNGS reports. We deeply appreciate the insightful comments and suggestions provided by JX, LZ, and LY, who greatly contributed to improving this paper. We thank LetPub (www.letpub.com.cn) for its linguistic assistance during the preparation of this paper.

Author Contributions

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

This study was supported by a grant from the National Key Research and Development Program of China (2023YFC2412900).

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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