Infection and Drug Resistance

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ORIGINAL RESEARCH

Diagnostic Performance of Bronchoalveolar Lavage Fluid Metagenomic Next-generation Sequencing versus Conventional Microbiological Tests in Immunocompromised and Immunocompetent Patients with Pulmonary Infection

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Purpose: Pulmonary infection in immunocompromised patients is challenging to diagnose. This study evaluated the detection performance of bronchoalveolar lavage fluid (BALF) metagenomic next-generation sequencing (mNGS) in these patients.

Patients and Methods: A total of 86 patients with confirmed pulmonary infection (48 immunocompromised, 38 immunocompetent) were retrospectively enrolled. BALF samples were tested by both mNGS and conventional microbiological tests (CMTs). Pathogen detection rates and pathogen spectrum were compared between the two groups.

Results: The positive rate of mNGS was significantly higher than that of CMTs in overall patients (87.21% vs 50.00%, $P < 0.001$), with a more prominent advantage in the immunocompromised group (97.92% vs 43.75%, $P < 0.001$). Detection rates of fungi (39.58% vs 5.26%, $P < 0.001$) and viruses (54.17% vs 28.95%, $P = 0.018$) were significantly higher in the immunocompromised group. Opportunistic pathogens including Pneumocystis jirovecii, cytomegalovirus, and Epstein-Barr virus were detected exclusively in the immunocompromised group. The mixed infection rate was also significantly higher in this group (72.92% vs 36.84%, $P < 0.001$).

Conclusion: BALF-mNGS is a reliable supplementary tool for conventional detection. It effectively identifies missed opportunistic pathogens and complex mixed infections, especially in immunocompromised patients. Combined application of mNGS and conventional tests improves pathogen detection and may help inform anti-infective treatment decisions.

Keywords: pulmonary infection, metagenomic next-generation sequencing, immunocompromised host, bronchoalveolar lavage fluid, mixed infection

Introduction

In recent years, the population of immunocompromised individuals has increased. This increase has been driven by the widespread use of chemotherapy for malignant tumours, long-term glucocorticoid therapy, and immunosuppressive agents. Immunocompromised status now represents a major clinical challenge.[1,2] Pulmonary infection is the most common complication in these patients, characterised by insidious onset, rapid progression, critical illness, and complex pathogens. It is therefore one of the leading causes of hospitalisation and death among immunocompromised hosts.[3] Because immune responses are impaired, clinical manifestations after infection are often atypical. Imaging findings are usually nonspecific. Etiological diagnosis is difficult. As a result, treatment is frequently delayed.[4]

https://doi.org/10.2147/IDR.S632502
Received: 17 June 2026
Accepted: 14 September 2026
Published: 22 September 2026

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Rapid and accurate pathogen identification is essential for improving outcomes in immunocompromised patients with pulmonary infections. At present, routine clinical microbiological methods mainly include bacterial culture, antigen–antibody assays, and polymerase chain reaction (PCR). However, these methods have several limitations. Culture requires a long turnaround time and has a low positivity rate. These limitations are more evident for fastidious organisms and in patients who have already received antibiotics. PCR has a limited detection range and cannot cover unknown or rare pathogens. Antigen–antibody assays have limited sensitivity and specificity. They are often inadequate for the diagnosis of mixed infections and opportunistic pathogens.[5–7] As such, there is an urgent need for a rapid, comprehensive, and highly sensitive pathogen detection technology to optimise the etiological diagnostic strategies for such patients.

Metagenomic next-generation sequencing (mNGS) is a high-throughput sequencing technology. Preset pathogen targets are not required. All nucleic acids in clinical samples can be sequenced directly. Potential pathogens, including bacteria, fungi, viruses, and parasites, can therefore be detected in a single assay.[8,9] In recent years, mNGS has demonstrated significant clinical application value in fields such as pulmonary infections, central nervous system infections, and bloodstream infections.[10–12] Compared with traditional methods, mNGS offers advantages such as a shorter detection cycle, broader pathogen spectrum coverage, and unique efficacy in diagnosing rare and mixed infections.[13] In immunocompromised patients, mNGS may overcome key limitations of conventional detection methods. Early pathogen identification and precise etiological diagnosis may therefore be achieved. However, mNGS also has inherent limitations, including the potential for background or colonizer contamination, high cost, reliance on bioinformatics analysis, and the lack of a universally standardized positivity threshold.[8] The interpretation of mNGS results requires careful differentiation between pathogenic and colonizing organisms.

In the present study, clinical data from patients with pulmonary infections were retrospectively analysed. The pathogen detection performance of mNGS and conventional methods was compared. Pathogen spectra and mixed-infection patterns were analysed between immunocompromised and immunocompetent groups. The detection performance of mNGS for diagnosing pulmonary infections in immunocompromised hosts was also explored. The aim of the study was to provide evidence for etiological diagnosis in these patients.

Materials and Methods

Study Subjects

Clinical data were collected from patients with pulmonary infection. All patients were hospitalised in the Department of Respiratory Medicine or the Department of Haematology of our hospital between January 2023 and December 2025. Pulmonary infection was diagnosed according to relevant guidelines and expert consensus for community-acquired pneumonia.[14,15] The final clinical diagnosis was jointly determined by a multidisciplinary team consisting of two senior respiratory physicians, one clinical microbiologist, and one radiologist, based on comprehensive assessment of clinical manifestations, imaging features, laboratory findings, and treatment response.

The inclusion criteria were as follows: (1) age ≥18 years; (2) fulfilment of the diagnostic criteria for pulmonary infection, with at least two of the following features: newly developed cough, sputum production, or aggravation of pre-existing respiratory symptoms; fever, defined as body temperature ≥38°C; signs of pulmonary consolidation or moist rales; peripheral white blood cell count $>10 \times 10^9$/L or $<4 \times 10^9$/L; or new exudation, consolidation, or ground-glass opacities on chest imaging; (3) bronchoalveolar lavage fluid (BALF) collected and submitted for both conventional microbiological culture and mNGS; and (4) complete clinical and laboratory data.

The exclusion criteria were as follows: (1) repeated samples from the same patient, for which only the first test result was included; (2) failed mNGS sequencing due to poor sample quality; and (3) missing key clinical information that prevented determination of immune status.

In our institutional practice, BALF mNGS testing was performed based on standardized clinical indications, including relatively severe pulmonary infiltrates, poor response to empirical anti-infective therapy, or clinical suspicion of opportunistic or rare pathogens. These criteria were applied uniformly to both immunocompromised and immunocompetent groups.

Mixed infection was defined as the identification of two or more clinically significant pathogens, determined by two senior physicians based on comprehensive assessment of clinical manifestations, imaging features, inflammatory markers, immune status, and treatment response. Microorganisms detected by mNGS were only considered clinically significant if they were judged to be pathogenic after integrating clinical data; colonizers, contaminants, and bystander organisms (eg, EBV without evidence of active infection) were excluded from the definition. When quantitative criteria were inconsistent with clinical judgment, the clinical comprehensive interpretation prevailed.

The present study was approved by the Medical Ethics Committee of Zhengzhou Central Hospital (approval no. ZXYY2026132). All patients provided informed consent for the use of their clinical data and biological samples in this retrospective study. The study is in line with the Declaration of Helsinki.

Case Source

All cases were derived from hospitalised patients in the Department of Respiratory Medicine and the Department of Haematology of our hospital. Patients were screened according to unified inclusion and exclusion criteria. Immune status was classified according to predefined criteria for immunocompromised status, as described in grouping method. Grouping was independent of the department of origin.

Grouping Method

Patients with confirmed pulmonary infection were divided into two groups according to immune status.

The immunocompromised group was defined by the presence of any of the following conditions: (1) primary immunodeficiency disease; (2) active malignancy, including solid tumours or hematologic malignancies, with ongoing chemotherapy or radiotherapy; (3) long-term use of immunosuppressive agents for >2 weeks, including glucocorticoids equivalent to prednisone ≥20 mg/day, a cumulative glucocorticoid dose >600 mg, or other immunosuppressive agents such as calcineurin inhibitors, biological immunomodulators, and antirheumatic drugs; (4) history of solid organ transplantation or hematopoietic stem cell transplantation; (5) HIV infection; and (6) diabetes mellitus with severe complications, such as diabetic ketoacidosis, stage V diabetic nephropathy, or diabetic foot with infection.

The immunocompetent group was defined as having none of the aforementioned conditions or having underlying diseases in a stable phase without the need for immunosuppressive therapy.

Method

Clinical Specimen Collection and Processing

Bronchoscopy was performed in all patients after hospital admission. BALF samples were collected according to standard operating procedures. Immediately after collection, each BALF sample was divided equally into two aliquots. One aliquot was placed in a sterile container and immediately submitted for routine microbiological testing. The other aliquot was placed in a sterile, nuclease-free, sealed container and immediately submitted for mNGS testing. If nucleic acid extraction could not be performed promptly, the sample was stored at −80°C. Conventional testing and mNGS were performed in parallel for each sample to reduce intergroup heterogeneity.

Conventional Microbiological Tests (CMTs)

BALF smear microscopy and bacterial culture were performed as baseline tests in all patients. Additional tests—including quantitative real-time PCR, Xpert MTB/RIF, G test, GM test, and T-SPOT.TB—were ordered at the clinicians' discretion based on comprehensive assessment of imaging findings, clinical manifestations, prior pathogen exposure, and local epidemiological data. All CMTs and mNGS were performed on the same BALF specimen and submitted simultaneously. The criteria for ordering additional tests were consistent between the two groups. All available test results were retrospectively collected for the study.

For bacterial culture, collected BALF samples were inoculated onto blood agar plates, chocolate agar plates, MacConkey agar plates, and Sabouraud dextrose agar plates. The inoculated media were incubated at 35°C in a Thermo Scientific CO_2 incubator. Bacterial isolates were identified using the BD Phoenix 100 automated microbial identification system (BD, USA).

Culture positivity was defined as the detection of definite pathogenic microorganisms, such as *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*, or *Aspergillus* spp. Culture results reported as "normal flora growth" or "no bacterial growth" were considered negative.

For conventional microbiological tests, an overall positive result was defined as fulfilment of any of the following criteria, provided that the detected microorganism was judged to be pathogenic by the multidisciplinary team: (1) positive culture; (2) detection of typical bacteria, fungi, or acid-fast bacilli by smear microscopy; (3) positive quantitative real-time PCR or Xpert MTB/RIF; (4) G test or GM test results reaching the diagnostic threshold; or (5) positive T-SPOT.TB results with clinical and radiological evidence supporting tuberculosis infection. T-SPOT.TB positivity alone was not used as a definitive diagnostic criterion. Colonising microorganisms were not counted as positive.

mNGS

Nucleic acid extraction: A 0.5 mL BALF sample was placed into a 1.5 mL microcentrifuge tube containing 1 g of 0.5 mm glass beads and vigorously agitated on a horizontal vortex mixer at 2,800–3,200 rpm for 30 minutes. Subsequently, 0.3 mL of the supernatant was transferred into a new 1.5 mL microcentrifuge tube. DNA and RNA were co-extracted using the VisionMedical Technologies nucleic acid extraction kit (VisionMedical Technologies Co., Ltd., Guangzhou, China) according to the manufacturer's instructions. Nucleic acid concentration was quantified using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, MA, USA).

Library construction and sequencing: The extracted RNA was reverse transcribed into complementary DNA (cDNA) using the VisionMedical Technologies reverse transcription kit. The DNA and cDNA were mixed, and libraries were constructed using the VisionMedical Technologies metagenomic library preparation kit. Qualified libraries were sequenced on a Nextseq 550 platform (Illumina, San Diego, USA) with a single-end 75 bp (SE75) sequencing mode, achieving a target data output of ≥20 million reads per sample. Negative controls (sterile saline) and positive controls (mock microbial community standards) were included in each sequencing run.

Bioinformatics analysis: Raw sequencing data were subjected to quality control to remove low-quality reads (Q score cutoff ≥20), adapter sequences, and duplicate reads. High-quality reads were aligned to the human reference genome (hg38) using Burrows-Wheeler Alignment (BWA) to remove human host sequences. The remaining data were aligned to a local microbial genome database, which was constructed based on public databases such as NCBI RefSeq, containing 25,863 pathogen species (12,142 bacteria, 2680 fungi, 10,061 viruses, 654 parasites, 206 mycobacteria, and 120 mycoplasma/chlamydia).

Interpretation criteria for mNGS results: To exclude potential colonizers, the detected microorganisms were compared with an in-house background microbial database to exclude common oral/upper respiratory tract colonizers (eg, *Streptococcus spp., Neisseria spp., Veillonella spp., Rothia spp., coagulase-negative staphylococci*). Pathogens with complete reference genomes were classified at the species level. Genomic coverage breadth was not employed as an independent threshold for positive calls; pathogen identification was determined by the RPTM criteria combined with negative control and in-house background filtering. The remaining microorganisms were filtered using the following criteria: ① Bacteria and fungi: specific reads per ten million (RPTM) ≥ 20; ② Viruses: RPTM ≥ 3; ③ Special pathogens (including *Mycobacterium tuberculosis, Cryptococcus spp*., etc).: specific reads ≥ 1; ④ All pathogens must satisfy: not detected in negative controls, or sample reads ≥ 10-fold of negative control reads.

Statistical Analysis

Statistical analyses were performed using SPSS version 19.0. Continuous variables were tested for normality using the Shapiro–Wilk test. Normally distributed data were expressed as mean ± SD and analyzed using the independent samples *t*-test. Non-normally distributed data were presented as median (IQR) and compared using the Mann–Whitney *U*-test. Categorical variables were expressed as numbers (%) and compared using the χ^2-test or Fisher's exact test. The McNemar test was used to compare positivity rates between mNGS and conventional methods. Cohen's kappa coefficient was calculated to assess their agreement. The Bonferroni correction was applied for multiple comparisons. A two-tailed $P < 0.05$ was considered statistically significant.

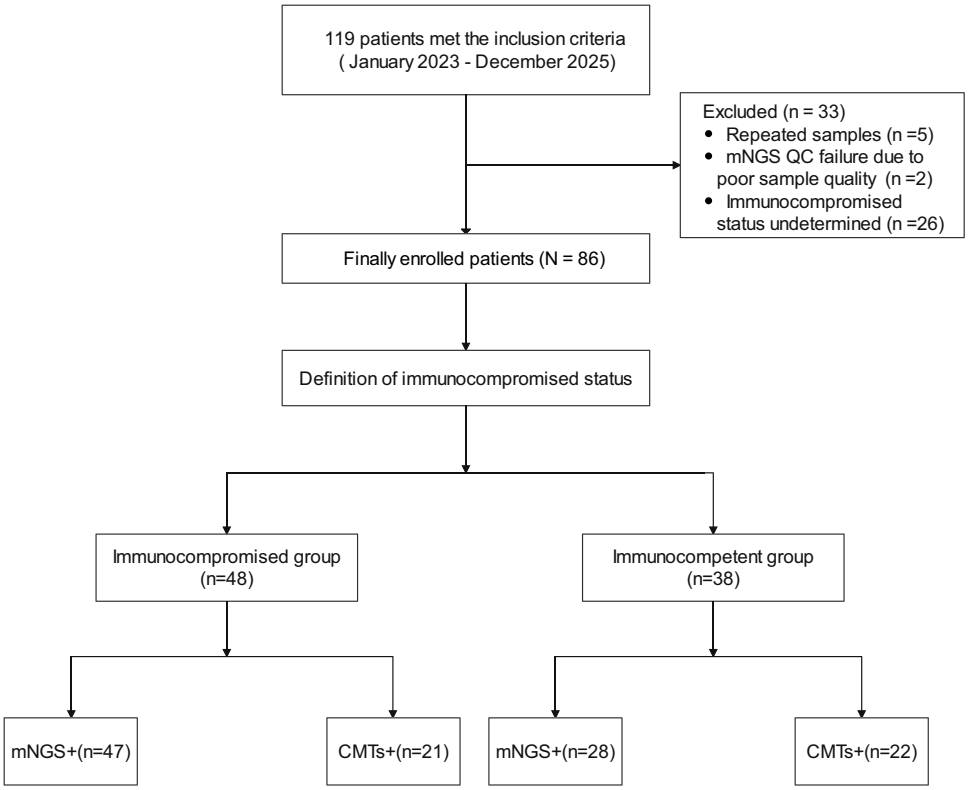

Figure 1 Study flowchart.

Results

Patient Screening and Baseline Characteristics

A total of 572 patients with suspected pulmonary infection were screened in this study. Based on the inclusion and exclusion criteria, 86 patients were finally enrolled in the analysis, including 48 (55.81%) in the immunocompromised group and 38 (44.19%) in the immunocompetent group. The detailed screening process is shown in Figure 1.

The baseline characteristics of the two groups are presented in Table 1. There were no statistically significant differences between the two groups in terms of age, sex distribution, history of antibiotic use before admission, or comorbidities (all $P > 0.05$). The distribution of immunosuppressive etiologies in the 48 immunocompromised patients was as follows: 34 cases

Table 1 Comparison of Clinical Characteristics Between the Two Groups

Characteristic	Immunocompromised Group (n=48)	Immunocompetent Group (n=38)	*P* value
Age (years), $\bar{x}\pm s$	61.96 ± 16.21	63.45 ± 14.44	0.658
Male [n(%)]	33 (68.75)	20 (52.63)	0.127
Antibiotic use before admission [n(%)]	20 (41.67)	14 (36.84)	0.650
Comorbidities [n(%)]			
Hypertension	19 (39.58)	17 (44.74)	0.631
Diabetes	11 (22.92)	9 (23.68)	0.935
Lung disease	9 (18.75)	8 (21.05)	0.790
Cardiovascular disease	13 (27.08)	13 (34.21)	0.471
Cerebrovascular disease	3 (6.25)	2 (5.26)	0.846
Clinical laboratory tests, median(IQR)			
WBC ($\times 10^9$/L), M (IQR)	6.00 (3.24, 8.89)	7.43 (5.48, 11.25)	0.036
CRP (mg/L), M (IQR)	37.68 (11.88, 84.56)	44.67 (7.90, 103.16)	0.801
PCT (ng/mL), M (IQR)	0.11 (0.07, 0.41)	0.06 (0.05, 0.12)	0.011

https://doi.org/10.2147/IDR.S632502

(70.83%) with active malignancy undergoing chemotherapy, including 28 cases (58.33%) of hematologic malignancy and 6 cases (12.50%) of solid tumor; 12 cases (25.00%) with long-term glucocorticoid use; and 4 cases (8.33%) with diabetes mellitus complicated by significant complications. Some patients had multiple immunosuppressive factors.

Regarding infection-related indicators, the white blood cell (WBC) count in the immunocompromised group was significantly lower than that in the immunocompetent group [6.00 (3.24, 8.89) vs 7.43 (5.48, 11.25), $P = 0.036$], which may be attributed to myelosuppression induced by chemotherapy or glucocorticoid therapy. In contrast, the procalcitonin (PCT) level was significantly higher in the immunocompromised group than in the immunocompetent group [0.11 (0.07, 0.41) vs 0.06 (0.05, 0.12), $P = 0.011$], suggesting a higher risk of bacterial infection in immunocompromised patients. No statistically significant difference was observed in C-reactive protein (CRP) levels between the two groups ($P > 0.05$).

Comparison of Detection Performance Between mNGS and CMTs

Among the 86 patients with confirmed pulmonary infection, the positive detection rate of mNGS was significantly higher than that of CMTs (87.21% vs 50.00%, $P < 0.001$). Stratified by immune status, the mNGS positive rate in the immunocompromised group (97.92%) was significantly higher than that of CMTs (43.75%) ($P < 0.001$); in the immunocompetent group, the mNGS positive rate (73.68%) was also higher than that of CMTs (57.89%) ($P = 0.039$) (Figure 2a).

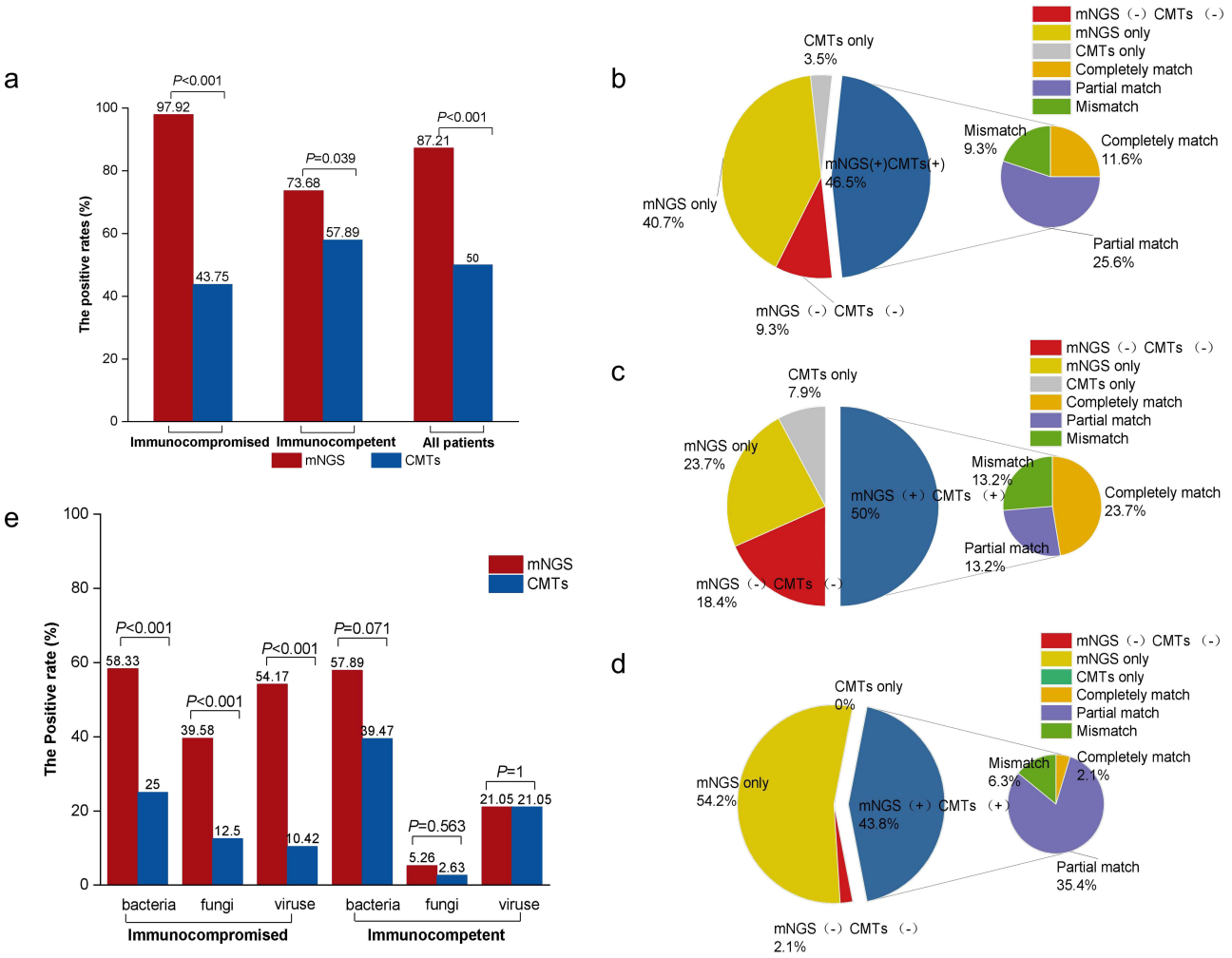

Figure 2 Comparison of detection performance between mNGS and CMTs. (**a**) Positive rates of mNGS and CMTs. (**b–d**) Consistency between the two methods in all patients (**b**) immunocompetent group (**c**) and immunocompromised group (**d**). (**e**) Comparison of positive rates stratified by pathogen category (bacteria, fungi, viruses). *P* values were adjusted using Bonferroni correction for multiple comparisons.

https://doi.org/10.2147/IDR.S632502

Consistency analysis showed an overall Kappa value of 0.116 ($P < 0.05$), indicating poor agreement between the two methods. Stratified consistency analysis revealed that mNGS-positive alone was the predominant type of discordance in both groups: 23.68% in the immunocompetent group and 54.17% in the immunocompromised group, with a significantly higher proportion in the immunocompromised group ($P = 0.005$). In contrast, the proportion of conventional-method-positive alone was extremely low (7.89% in the immunocompetent group and 0.00% in the immunocompromised group), suggesting a high rate of missed detection and prominent false negativity with conventional methods. The Kappa value was 0.033 in the immunocompromised group and 0.317 in the immunocompetent group, indicating that immune status affects the agreement between the two methods, with mNGS demonstrating a more pronounced advantage in immunocompromised patients (Figure 2b–d).

Among patients with positive results by both methods, the immunocompromised group predominantly showed partial concordance (80.95%, 17/21), with a complete concordance rate of only 9.52% (2/21); in contrast, the immunocompetent group had a complete concordance rate of 47.37% (9/19), significantly higher than that in the immunocompromised group. This suggests that the pathogen composition is more complex in immunocompromised patients, which conventional methods cannot fully capture, whereas mNGS can identify more potentially pathogenic microbial information (Figure 2b–d).

Further stratified analysis by pathogen type (Figure 2e) showed that in the immunocompromised group, mNGS significantly outperformed CMTs in detecting bacteria (58.33% vs 25.00%), fungi (39.58% vs 12.50%), and viruses (54.17% vs 10.42%) (all Bonferroni-adjusted $P < 0.001$). In the immunocompetent group, mNGS showed a higher bacterial detection rate than CMTs (57.89% vs 39.47%), but the difference was not significant ($P = 0.071$), and no significant differences were observed for fungi or viruses (both $P > 0.05$). These results further confirm that mNGS offers a greater advantage in immunocompromised patients, particularly in detecting fungi and viruses.

To assess whether non-standardized CMTs biased the results, we analyzed patients who received the complete CMTs panel (BALF smear, bacterial culture, quantitative PCR, G test, GM test, Xpert MTB/RIF). In the immunocompromised subgroup (n=29), mNGS positivity was significantly higher than CMTs [100% (29/29) vs 51.72% (15/29), $P < 0.001$]. In the immunocompetent subgroup (n=11), the difference was not significant [90.91% (10/11) vs 72.73% (8/11), $P = 0.625$]. These findings were consistent with the main analysis.

To further characterize the heterogeneity of the immunocompromised group, we performed a descriptive subgroup analysis stratified by immunosuppressive etiology. As shown in Table 2, the highest mixed infection rate was observed in patients with hematologic malignancies receiving chemotherapy (80.77%, 21/26), followed by solid tumors (66.67%, 4/6), long-term glucocorticoid use (60.00%, 6/10), and diabetes with severe complications (50.00%, 2/4). mNGS positivity rates consistently exceeded CMT positivity rates across all subgroups (hematologic malignancy: 100% vs 46.15%; solid tumor: 83.33% vs 50.00%; glucocorticoid: 100% vs 40.00%; diabetes: 100% vs 25.00%), indicating that the diagnostic advantage of mNGS over CMTs is consistent across different types of immunodeficiency.

Pathogen Spectrum Characteristics

Comparison of Pathogen Detection Rates Between the Two Groups

The pathogen detection profiles of the immunocompromised group and the immunocompetent group are shown in Figure 3a and b. The fungal detection rate in the immunocompromised group (39.58%) was significantly higher than that

Table 2 Subgroup Analysis of Immunocompromised Patients Stratified by Immunosuppressive Etiology

Subgroup	n	Mixed Infection Rate	mNGS Positivity	CMT Positivity
Hematologic malignancy on chemotherapy	26	80.77% (21/26)	100% (26/26)	46.15% (12/26)
Solid tumor on chemotherapy	6	66.67% (4/6)	83.33% (5/6)	50.00% (3/6)
Long-term glucocorticoid use	10	60.00% (6/10)	100% (10/10)	40.00% (4/10)
Diabetes with severe complications	4	50.00% (2/4)	100% (4/4)	25.00% (1/4)
Hematologic malignancy + long-term glucocorticoid use*	2	100% (2/2)	100% (2/2)	50.00% (1/2)
Total	48	72.92% (35/48)	97.92% (47/48)	43.75% (21/48)

Notes: *Patients with overlapping immunosuppressive factors were listed separately and not merged into single-etiology subgroups.

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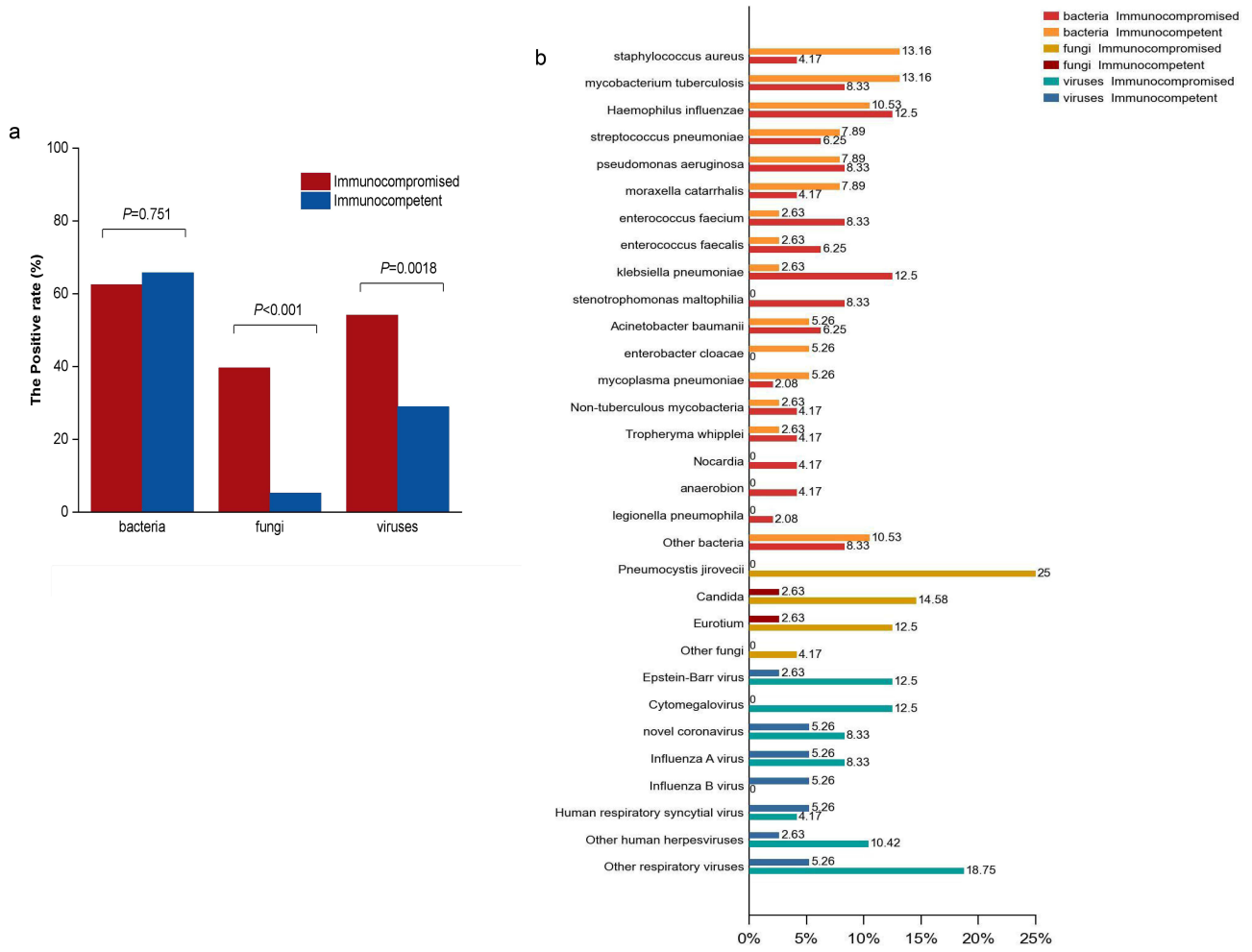

Figure 3 Pathogen profiles in immunocompromised and immunocompetent groups. (**a**) Comparison of bacterial, fungal, and viral detection rates between the two groups. (**b**) Distribution of major pathogens detected in both groups.

in the immunocompetent group (5.26%) ($P < 0.001$). Similarly, the viral detection rate (54.17%) was significantly higher in the immunocompromised group than in the immunocompetent group (28.95%) ($P = 0.018$). No statistically significant difference was observed in bacterial detection rates between the two groups (62.50% vs 65.79%, $P = 0.751$).

Distribution of Common Pathogens in the Two Groups

Bacterial infections predominated in both groups; however, the immunocompromised group exhibited significantly higher detection rates of fungi, viruses, and opportunistic pathogens, with a more complex pathogen spectrum and a higher prevalence of mixed infections.

Among bacterial pathogens, the immunocompromised group was characterized predominantly by opportunistic pathogens. The detection rates of *Klebsiella pneumoniae* (6/48 vs 1/38, $P = 0.049$), *Enterococcus faecium* (4/48 vs 1/38, $P = 0.037$), and *Stenotrophomonas maltophilia* (4/48 vs 0/38, $P = 0.042$) were significantly higher in the immunocompromised group than in the immunocompetent group. Opportunistic pathogens such as *Nocardia* spp., anaerobic bacteria, and *Legionella pneumophila* were detected exclusively in the immunocompromised group. *Staphylococcus aureus* was relatively more common in the immunocompetent group (5/38 vs 2/48, P = 0.093), although the difference did not reach statistical significance.

Regarding fungal pathogens, the detection rate of *Pneumocystis jirovecii* was significantly higher in the immunocompromised group (12/48 vs 0/38, $P < 0.001$). Additionally, *Candida* spp. (7/48 vs 1/38, $P = 0.038$) and *Aspergillus* spp. (6/48 vs 1/38, $P = 0.089$) were more frequently detected in the immunocompromised group.

https://doi.org/10.2147/IDR.S632502

Table 3 Distribution of Mixed Infection Patterns in Immunocompetent and Immunocompromised Groups [n (%)]

Mixed Infection Pattern	Immunocompetent (n = 38)	Immunocompromised (n = 48)
Multiple bacteria only	7 (18.42)	6 (12.50)
Multiple fungi only	0 (0.00)	1 (2.08)
Multiple viruses only	0 (0.00)	3 (6.25)
Bacteria + Fungi	1 (2.63)	5 (10.42)
Bacteria + Virus	6 (15.79)	12 (25.00)
Fungi + Virus	0 (0.00)	3 (6.25)
Bacteria + Fungi + Virus	0 (0.00)	5 (10.42)
Total mixed infections	14 (36.84)	35 (72.92)

In terms of viral pathogens, the immunocompetent group predominantly exhibited infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus, influenza B virus, and human respiratory syncytial virus (each approximately 5.26%), with rare detection of herpesviruses. In contrast, the immunocompromised group showed a broader viral spectrum and higher detection rates, with prominent proportions of Epstein–Barr virus (12.50%), cytomegalovirus (12.50%), and other respiratory viruses (18.75%). The detection rate of herpesviruses was significantly higher in the immunocompromised group than in the immunocompetent group, indicating an elevated risk of virus-associated opportunistic infections in immunocompromised patients.

Mixed Infection Analysis

The detection rate of mixed infections in the immunocompromised group was 72.92% (35/48), which was significantly higher than that in the immunocompetent group (36.84%, 14/38), with a statistically significant difference ($P < 0.001$). The distribution of mixed infection types in the two groups is presented in Table 3.

Regarding mixed infection patterns, the predominant patterns in the immunocompromised group were bacterial–viral co-infection (25.00%) and bacterial–fungal co-infection (10.42%), whereas bacterial–viral co-infection (15.79%) was the most common pattern in the immunocompetent group. The proportion of complex mixed infections involving three or more pathogen types was higher in the immunocompromised group than in the immunocompetent group (37.50% vs 18.42%). Moreover, triple infections involving bacteria, fungi, and viruses (10.42%) were observed exclusively in the immunocompromised group, suggesting that immunocompromised patients have a more complex pathogen spectrum and a greater burden of mixed infections.

mNGS Detection in Conventional Method-Negative Samples

Among the 43 patients with negative results by conventional methods, mNGS detected pathogens in 35 cases (81.40%). In the immunocompromised group, 27 patients tested negative by conventional methods, of whom 26 (96.30%) were positive by mNGS. In the immunocompetent group, 16 patients tested negative by conventional methods, of whom 9 (56.25%) were positive by mNGS. The difference between the two groups was statistically significant ($P = 0.002$). The main pathogens detected by mNGS in conventional method-negative samples were *Pneumocystis jirovecii* (6 cases), cytomegalovirus (5 cases), *Haemophilus influenzae* (5 cases), Epstein–Barr virus (4 cases), and *Aspergillus fumigatus* (3 cases).

Antimicrobial Regimen Changes and AMR Gene Findings from mNGS

We retrospectively reviewed antimicrobial regimen adjustments made within 72 hours after mNGS results were issued. As shown in Table 4, treatment modifications occurred in 14 of 38 patients (36.84%) in the immunocompetent group and 28 of 48 patients (58.33%) in the immunocompromised group. Escalation or addition of targeted therapy was the most frequent modification (immunocompetent: 28.95%, 11/38; immunocompromised: 50.00%, 24/48). In the immunocompromised group, additions of anti-Pneumocystis therapy (16.67%, 8/48), antifungal therapy (10.42%, 5/48), and antiviral

Table 4 Treatment Modifications Within 72 hours Following mNGS Reporting

Adjustment Type	Immunocompetent (n=38)	Immunocompromised (n=48)
Escalation/Addition	11 (28.95%)	24 (50.00%)
Anti-Pneumocystis (TMP-SMX)	0 (0%)	8 (16.67%)
Antifungal (voriconazole, etc.)	1 (2.63%)	5 (10.42%)
Antiviral (ganciclovir, etc.)	1 (2.63%)	4 (8.33%)
Antituberculous	4 (10.53%)	2 (4.17%)
Antibacterial/other	4 (10.53%)	5 (10.42%)
Switching	3 (7.89%)	2 (4.17%)
De-escalation/Discontinuation	1 (2.63%)	1 (2.08%)
No change	24 (63.16%)	20 (41.67%)
Total modification	14 (36.84%)	28 (58.33%)

therapy (8.33%, 4/48) were particularly prominent, consistent with the higher detection rate of opportunistic pathogens in this population. De-escalation or discontinuation of antibiotics was uncommon in both groups (immunocompetent: 2.63%; immunocompromised: 2.08%).

AMR genes were detected in 10 patients, including 8 in the immunocompromised group (16.67%, 8/48) and 2 in the immunocompetent group (5.26%, 2/38). The predominant genes were β-lactamase genes (blaTEM, blaSHV, blaCTX-M, blaKPC) in 8 cases, among which one immunocompromised patient co-carried a carbapenemase gene (blaKPC) together with multiple ESBL genes (blaCTX-M, blaSHV, blaTEM). Ribosomal target modification genes (ermB, ermC) were detected in 3 cases. Phenotypic susceptibility testing confirmed concordance with genotype in all AMR gene-positive isolates.

Discussion

Pulmonary infection is the most common complication with a poor prognosis in immunocompromised individuals.[16] In this population, the immune defence system is significantly impaired, and the clinical symptoms at the onset of infection are often insidious, lacking typical manifestations of infection.[17] Nonetheless, disease progression is rapid. Severe pneumonia may develop within a short period. The clinical case fatality rate is high.[3] Traditional etiological diagnostic methods, including bacterial culture, serological testing, and PCR, generally have inherent limitations such as long detection cycles, low positive rates, and limited pathogen coverage.[5–7] In immunocompromised patients, infections are complex and heterogeneous. Conventional methods often fail to provide timely and accurate etiological evidence. As a result, early precision anti-infective therapy may be delayed or restricted. mNGS is an unbiased, high-throughput sequencing technology. Microbial nucleic acids in clinical samples can be detected without prespecified targets. Broad pathogen coverage and high sensitivity can therefore be achieved.[8,9] BALF was selected as the detection specimen, which can directly obtain information on in situ pathogenic microorganisms in the lower respiratory tract.[18] Compared with sputum, BALF is less affected by contamination from upper respiratory tract flora. Therefore, the pathogen composition of local pulmonary infection may be reflected more objectively and accurately. BALF may provide higher clinical diagnostic value.[19] Systematic review by Chen et al[19] confirmed that BALF-mNGS outperforms conventional microbiological methods for the diagnosis of pulmonary infections, especially in immunocompromised patients. However, the existing evidence lacks detailed immune-status-stratified comparisons, individualized pathogen spectrum characteristics, and clinical practice implications. Against this background, we retrospectively analyzed BALF-mNGS results and clinical data from 86 patients with pulmonary infection. We systematically compared the pathogen detection performance of mNGS and CMTs between immunocompromised and immunocompetent patients, and further characterized the distribution patterns of pathogenic microorganisms and mixed infection profiles across different immune statuses and immunosuppressive etiologies. In addition, we evaluated post-mNGS treatment modifications and antimicrobial resistance gene findings to explore the potential clinical value of mNGS. This study aimed to provide stratified evidence for optimizing pathogen detection strategies in immunocompromised patients with pulmonary infection.

The results of the study show that the total positive detection rate of mNGS was significantly higher than that of CMTs (87.21% vs 50.00%, $P < 0.001$), with a more prominent advantage in the immunocompromised group (97.92% vs 43.75%, $P < 0.001$). This is consistent with previous research findings.[20,21] Agreement analysis showed extremely poor concordance between the two methods in immunocompromised patients. The Kappa value was only 0.033. In this group, mNGS-positive-only cases accounted for 54.17%. No cases were positive only by CMTs. These findings indicate that substantial pathogen underdetection occurs when CMTs are used in immunocompromised patients. Stratified analysis by pathogen type revealed that the detection rates of fungal and viral pathogens by CMTs in the immunocompromised group were only 12.50% and 10.42%, respectively, whereas mNGS achieved rates of 39.58% and 54.17% (both $P < 0.001$). These results suggest that superior detection of fungal and viral pathogens was the main reason for the higher overall positivity rate of mNGS. Thus, mNGS may be particularly suitable for pathogen detection in immunocompromised patients.

The present study shows that pulmonary infections in immunocompromised patients had distinct etiological characteristics. First, the detection rate of opportunistic pathogens was significantly increased. *Pneumocystis jirovecii* (25.0%), cytomegalovirus (12.5%), and Epstein–Barr virus (12.5%) were detected only in the immunocompromised group. These findings are consistent with cellular immune deficiency and latent viral reactivation in this population. Second, fungal infections were common. *Aspergillus* spp. (12.5%) and *Candida* spp. (14.6%) were detected more frequently in the immunocompromised group. Third, bacterial infections were mainly caused by opportunistic pathogens. This pattern may be related to prolonged hospitalisation, repeated exposure to broad-spectrum antibiotics, and immunosuppression.[22] Compared with previous studies, the detection rate of *P. jirovecii* in the present study (25.0%) was higher than the 7.84% reported by Zhao et al.[23] This difference may be explained by the higher proportion of patients with hematologic malignancies in the present study (58.33%). These patients may have more severe cellular immune deficiency after chemotherapy.

The co-infection rate in the immunocompromised group was 72.92%, significantly higher than that in the immunocompetent group (36.84%, $P < 0.001$). Bacterial–viral co-infections were the most common pattern (25.00%), and triple infections involving bacteria, fungi, and viruses (10.42%) were detected exclusively in the immunocompromised group. Such polymicrobial infections substantially complicate clinical diagnosis and treatment, as a single empirical anti-infective regimen is often insufficient to cover all pathogens. Narrow-spectrum regimens may result in inadequate pathogen coverage, while injudicious broad-spectrum antimicrobial use promotes MDRO emergence. A risk-stratified approach is therefore recommended—initial therapy should be guided by immune status, imaging features, prior MDRO exposure, and local epidemiological resistance data, with timely antimicrobial de-escalation after mNGS results become available. Furthermore, given the high analytical sensitivity of mNGS, positive findings—particularly for fungi (*Aspergillus* and *Pneumocystis jirovecii*), respiratory viruses, and commensal bacteria—must be interpreted with caution. Correlation with clinical manifestations, radiological infiltrates, inflammatory biomarkers, and host immune status is essential to distinguish true infection from colonization, thereby avoiding unnecessary antimicrobial exposure and reducing selective pressure for resistance. mNGS can detect multiple pathogens in a single assay and provide evidence that may inform anti-infective treatment decisions. Therefore, in immunocompromised patients with pulmonary infection, a high level of suspicion for co-infection should be maintained, and mNGS may serve as an important supplement to conventional methods—particularly in patients with negative conventional test results or suspected infections caused by unusual pathogens.

In the present study, the WBC level in the immunocompromised group was significantly lower than that in the immunocompetent group ($P = 0.036$). This finding may be related to bone marrow suppression caused by chemotherapy or glucocorticoid therapy. It also suggests that white blood cell count has limited value for infection assessment in immunosuppressed patients. In contrast, procalcitonin levels were significantly increased in the immunocompromised group. This finding supports the value of procalcitonin as a serological marker for bacterial infection screening and severity assessment in this population, consistent with previous studies.[24] There was no significant difference in CRP levels between the two groups ($P = 0.801$), possibly due to its low inflammatory specificity and susceptibility to interference from various non-infectious factors in the body.[25]

Subgroup analysis using a complete CMTs panel confirmed the robustness of our main findings, ruling out the possibility that the superior performance of mNGS in immunocompromised patients was driven by non-standardized CMTs testing (100% vs 51.72%, $P < 0.001$). Although CMTs positivity increased from 43.75% in the overall cohort to 51.72% in the complete-testing subgroup, mNGS remained significantly superior after this bias was minimized. In immunocompetent patients, no significant difference was detected, which may be attributable to the small sample size (n=11) and the satisfactory performance of conventional tests in this population.

Several limitations should be acknowledged. First, this was a single-centre retrospective study. The strict inclusion criteria contributed to the limited sample size and may have led to cohort enrichment, potentially influencing the detection rates of mixed infections and rare pathogens. Future prospective multicentre studies are warranted to validate these findings. Second, among the conventional methods, only culture was performed in all patients. Auxiliary tests, including PCR and antigen detection, were selectively performed according to clinical need. This resulted in heterogeneity in conventional testing and may have affected the accuracy of the positivity rate for conventional methods. Third, the effect of mNGS on long-term clinical outcomes was not evaluated in this retrospective study, and AMR gene detection was exploratory with a limited number of positive cases (n=10). Prospective studies are needed to further investigate these outcomes.

Conclusion

In conclusion, mNGS can serve as an important supplement to conventional methods for the etiological diagnosis of pulmonary infections. Its auxiliary diagnostic value is particularly evident in immunocompromised patients. Pulmonary infections in these patients have distinct etiological characteristics. Opportunistic pathogens are frequently detected. The co-infection rate is also significantly increased. These features make clinical diagnosis more difficult. BALF-based mNGS enables comprehensive screening for rare, difficult-to-diagnose pathogens. The pathogen spectrum in immunocompromised patients can therefore be more clearly defined. Reliable pathogen detection evidence can also be provided for the early development of individualised anti-infective treatment strategies.

Acknowledgments

The authors thank the Department of Hematology and the Department of Clinical Laboratory at Zhengzhou Central Hospital for providing clinical data and supporting this study.

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 work was supported by the Training Program for Improving the Respiratory Interventional Diagnosis and Treatment Capacity of Central Airway Lesions in Primary Care Units (P-20251022-0206).

Disclosure

The authors report no conflicts of interest in this work.

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