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The clinical application of metagenomic next-generation sequencing in immunocompromised patients with severe respiratory infections in the ICU

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Abstract

Background Early targeted antibiotic therapy is crucial for improving the prognosis of immunocompromised patients with severe respiratory infections (SRIs) in the intensive care unit (ICU). Metagenomic next-generation sequencing (mNGS) has shown significant value in pathogen detection, but research on lower respiratory tract microorganisms remains limited.

Methods This study enrolled 234 patients with SRIs in the ICU, and individuals were categorized into immunocompromised and immunocompetent groups. We compared the diagnostic performance of mNGS using bronchoalveolar lavage fluid (BALF) with conventional microbiological tests (CMTs) and analyzed the value of mNGS in immunocompromised patients with SRIs in the ICU.

Results Among all patients, the pathogenic microorganism detection rate of mNGS was higher than that of CMTs (94.02% vs 66.67%, $P<0.05$), both in the immunocompromised group (95.0% vs 58.75%, $P<0.05$) and the immunocompetent group (93.51% vs 71.43%, $P<0.05$). mNGS detected more pathogens than CMTs did (167 vs 51), identifying 116 organisms that were missed by CMTs. The proportion of antibiotic regimen adjustments based on mNGS results was significantly higher compared to CMTs in both the immunocompromised (70.00% vs 17.50%, $P<0.05$) and immunocompetent groups (48.70% vs 15.58%, $P<0.05$). In the immunocompromised group, patients who had their antibiotic treatment adjusted on mNGS results had improved prognosis, with significantly lower ICU mortality (8.93% vs 50%, $P<0.05$) and 28-day mortality rates (30.36% vs 68.75%, $P<0.05$) than CMTs. In the immunocompetent group, no statistically significant differences were observed in ICU mortality or 28-day mortality (20.00% vs 33.33%, $P>0.05$; 42.67% vs 45.83%, $P>0.05$).

Conclusion mNGS shows significant value in detecting pathogens in immunocompromised patients with SRIs in ICU. For immunocompromised patients who respond poorly to empirical treatment, mNGS can provide an etiological basis, helping adjust antibiotic regimens more precisely and thereby improving patient prognosis.

Keywords Antibiotics, Etiology, Metagenomic next-generation sequencing, Severe respiratory infections, Bronchoalveolar lavage fluid

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Introduction

An increasing number of adults have immune dysfunction, and the proportion of immunocompromised patients in the intensive care unit (ICU) is increasing. According to statistics, approximately one-third of ICU patients have at least one risk factor for immunosuppression [1]. Repeated severe respiratory infections (SRIs) are one of the primary reasons immunocompromised patients are admitted to the ICU [2]. Compared with immunocompetent patients, these patients are more prone to developing complex infections involving bacteria, fungi, viruses, and rare parasites. This can lead to hypoxemic acute respiratory failure (ARF) and sepsis, significantly impacting patient prognosis [3]. Early detection of pathogens in immunocompromised patients with SRIs is crucial for guiding clinical interventions and administering appropriate targeted antibiotic therapy [4], which is essential for improving their prognosis.

However, conventional pathogen detection methods, such as bacterial smears of sputum or bronchoalveolar lavage fluid (BALF), culture, or nucleic acid detection of pharyngeal swabs, are limited by the diversity of pathogens, the heterogeneity of sampling, and the constraints of the detection methods themselves [5]. These factors make identifying lower respiratory tract pathogens challenging, and the results are often not accurate enough. Research indicates that approximately 40–60% of pathogens in the clinic are undiagnosed [6, 7], preventing clinicians from adjusting antibiotic treatment promptly on the basis of the responsible pathogen, thereby potentially worsening the condition and increasing the risk of mortality.

Metagenomic next-generation sequencing (mNGS), a novel pathogen detection technology, has been widely applied in clinical settings. It offers advantages such as high efficiency, a broad pathogen spectrum, and high sensitivity, making it particularly suitable for detecting rare or emerging pathogens [8, 9]. Additionally, mNGS has demonstrated superior diagnostic performance compared with conventional methods [10, 11]. Zhou et al. reported that, compared with conventional methods, mNGS has a higher diagnostic rate for pathogen detection in patients who have received antibiotic treatment [12]. Multiple studies have also shown that mNGS has significant value in the diagnosis, treatment, and prognosis of severe SRIs [13–15]. Compared with conventional methods, mNGS can identify pathogens more quickly and accurately, allowing clinicians to adjust antibiotic regimens earlier to target the causative bacteria, thereby significantly reducing the in-hospital mortality rate of patients with SRIs.

However, comprehensive research on the application of mNGS in the diagnosis of SRIs remains scarce. To address this gap, we conducted a study to compare the diagnostic efficiency of conventional methods and mNGS for ICU immunocompromised patients with SRIs and to evaluate the clinical impact of mNGS on these patients.

Materials and methods

Ethical approval and consent

This research was approved by the ethics committee of Quzhou People's Hospital (Quzhou People's Hospital, Quzhou, China: Number B 2024-092). Informed consent was obtained from all patients or their next of kin.

Study design and participants

This retrospective study included 322 ICU patients with clinical suspicion of SRIs who were admitted to Quzhou People's Hospital affiliated with Wenzhou Medical University from January, 2022, to March, 2024. Based on the inclusion/exclusion criteria, 234 patients were included in the study (Fig. 1).

The inclusion criteria were as follows: (1) aged ≥ 18 years; (2) had an initial diagnosis of SRIs and used antibiotics during hospitalization; and (3) underwent routine conventional microbiological tests (CMTs) and mNGS at the same time.

The exclusion criteria were as follows: (1) did not agree to undergo mNGS detection; (2) had unqualified specimens and incomplete clinical data; (3) CMTs and mNGS were not tested simultaneously; and (4) had an unknown prognosis within 28 days.

The diagnostic standard of SRIs were as follows [2]: (1) ICU admission and/or receiving mechanical ventilation due to respiratory infections; (2) unabating high fever, disturbance of consciousness, hypoxemia, cyanosis, dyspnea; (3) radiological confirmation of multilobar involvement, pleural effusion.

The diagnostic criteria of pulmonary mycosis were as follows [16] (at least one): (1) abnormal chest radiographic images suggestive of pulmonary mycosis, (2) identification of fungal genera or species in sputum culture or smear-positive, and (3) positive GM test in serum, BALF or mNGS. Normal flora of the respiratory tract was not interpreted as pathogens.

Patients were divided into an immunocompromised group and an immunocompetent group according to the following definitions of immunocompromised status [17]:

(1). Hematological malignancy (active or in remission for less than 5 years);
(2). Solid organ transplantation or hematopoietic stem cell transplantation;
(3). Neutropenia or chemotherapy for solid tumors in the past 3 months;

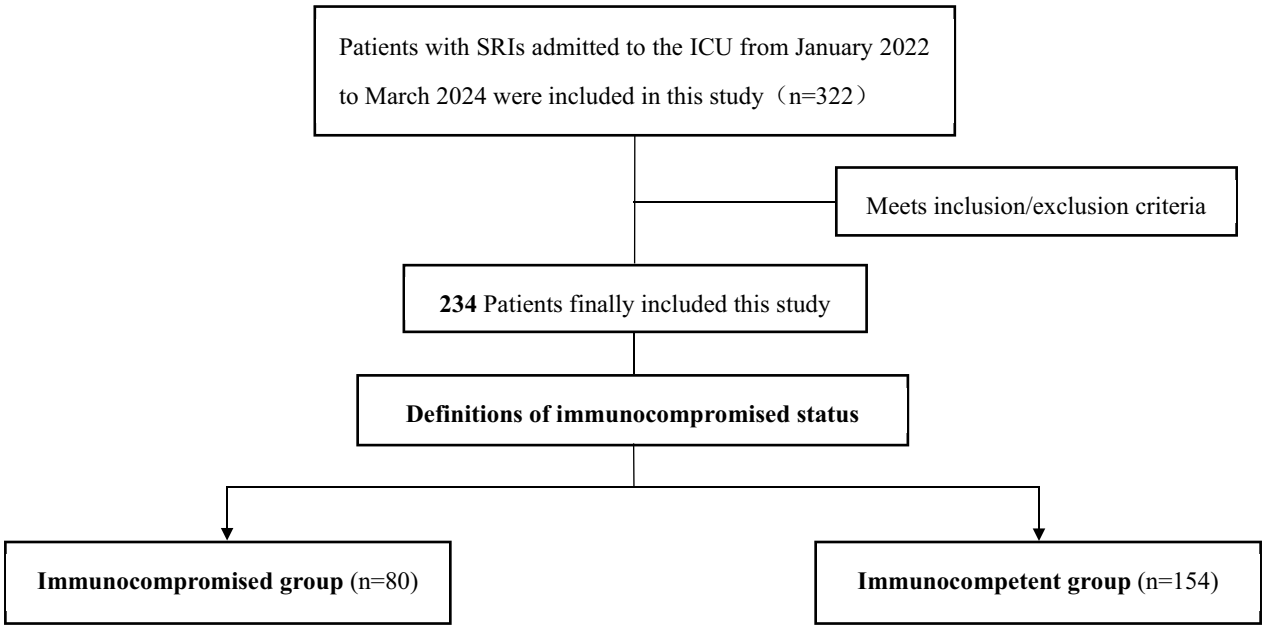


Fig. 1 Study flow diagram

(4). Use of immunosuppressants, biological immunomodulators, and antirheumatic drugs (e.g., methotrexate, cyclophosphamide, and cyclosporin);
(5). Daily intake of more than 20 mg of glucocorticoids for more than 14 days (or a cumulative dose of 700 mg prednisolone, or equivalent doses of other corticosteroids);
(6). Immunocompromised status due to hereditary or congenital factors.

Conventional microbiological tests

Pathogenic microorganisms identified through CMTs include blood cultures, sputum cultures, bronchoalveolar lavage fluid (BALF) cultures, and polymerase chain reaction (PCR). Following the exclusion of contraindications, bronchoscopy is performed by a trained physician under sterile conditions to collect BALF, which is promptly processed for microbiological analysis. PCR is primarily used for viral detection and specific pathogen identification. Additionally, chest computed tomography (CT) and other imaging modalities are employed to assist in the overall diagnostic process.

The mngs procedure

Sample processing and DNA/RNA extraction

According to standard operations, BALF samples (1.5–3 ml) were collected from patients via a bronchoscope. BALF samples were centrifuged at 12,075 × g and 4 °C for 5 min. For each sample, 500 μL of supernatant was used to extract genomic DNA via the PathoXtract® WYXM03202S Universal Pathogen Enrichment Extraction Kit (WillingMed, Beijing, China), and RNA was extracted using PathoXtract® Virus DNA/RNA Isolation Kit (WYXM03009S, WillingMed Corp, Beijing, China) according to the manufacturer's protocol.

Construction of DNA/RNA libraries and sequencing

For only DNA pathogen detection, an Illumina® DNA Prep (M) Tagmentation Kit (20018705; Illumina, San Diego, USA) was used to construct the DNA libraries. For DNA and RNA pathogen co-detection, DNA and RNA were mixed and then reverse transcription of the RNA to complementary DNA (cDNA) was performed by using SuperScript® Double-Stranded cDNA Synthesis Kit (11917020, Invitrogen). The quality of the libraries was assessed using a Qubit fluorescence quantification analyzer (Thermo Fisher) and an Agilent 2100 Bioanalyzer (Agilent Technologies). Sequencing was performed on a NextSeq™ 550Dx sequencer (Illumina, San Diego, USA), with each sample achieving at least 20 million sequencing reads.

Bioinformatic analysis

Sequencing data were processed automatically to produce a detection report. The FASTQ-format data obtained from sequencing were processed with Trimmomatic v0.40 [18] to eliminate low-quality or undetected sequences, splice contaminants, high-coverage repeats,

and short-read-length sequences. The high-quality sequencing data were then compared to the human reference genome GRCh37 (hg19) via Bowtie2 v2.4.3 [19] to remove human host sequences. The remaining sequences were aligned to a previously constructed reference database containing more than 24,000 pathogens via Kraken2 v2.1.0 [20] for pathogen identification. The number of species-specific reads identified was normalized to the number of reads per ten million (RPTM) to determine positive results.

Criteria for a positive mngs result

(1). The total number of sample sequences was greater than or equal to 20 million reads.
(2). The ratio of the reads per million sample divided by the reads per million of the no-template controls from any given taxon (species, genus, or family) ≥ 10 [21, 22].
(3). Bacteria (excluding mycobacteria), viruses, and parasites: mNGS identified a microbe (species level) whose coverage rate was tenfold greater than that of any other microbe [23].
(4). Fungi: mNGS identified a microbe (at the species level) whose coverage rate was fivefold higher than that of any other fungus because of its low biomass during DNA extraction [24].

Evaluation of the clinical impact of mngs or cmts on antibiotic regimens adjustments and patient prognosis

In this study, pathogen identification was independently conducted by a panel of two senior physicians. The diagnosis was based on a comprehensive assessment including clinical presentation, laboratory tests, mNGS results, imaging studies, and adjustments to patient management. Any disagreements among clinicians were resolved through further discussion and consensus.

First, we compared the impact of adjustments in antibiotic regimens and patient prognosis based on mNGS or CMTs results. To further assess the clinical effects of mNGS in immunocompromised group, we categorized its clinical effects into positive, negative, and no effect. Positive effects were defined as mNGS results that contributed to definitive diagnosis and led to antibiotic regimen adjustments, such as antibiotic changed; escalation or de-escalation of antibiotics; adjunctive, antiviral or antituberculosis treatments. Additionally, it included the continuation of empirical antibiotic treatment. Negative effects were defined as mNGS results led to incorrect diagnoses, resulting in incorrect antibiotic treatment. No clinical effects were defined as negative mNGS results or insignificant mNGS findings (identified as non-pathogenic organisms or normal respiratory flora/colonizers).

The evaluation time point set at 72 h after obtaining the mNGS results to assess whether the findings led to antibiotic regimen adjustments of patient (positive effects, negative effects or no clinical effects).

Statistical analysis

The data were statistically analyzed via SPSS 26.0 and R software (v4.4.1), and figures were created via GraphPad Prism 10 software. Continuous variables were tested for normality via the Kolmogorov–Smirnov test and are expressed as medians and interquartile ranges (IQR) or as means ± SDs, depending on the distribution. Data comparisons were performed via an independent samples t test or the Mann–Whitney U test. Categorical variables were compared via the chi-square test. The kappa consistency test was conducted to determine whether the diagnostic results of the two methods were consistent. The R packages circlize and ComplexHeatmap were used to analyze the detection rates of pathogens by mNGS and CMTs. In this study, $P < 0.05$ was considered statistically significant.

Results

Distribution of immunocompromised patients and patient characteristics

According to the inclusion/exclusion criteria, 234 patients with SRIs, including 117 males and 117 females, were included in this analysis. The patients were divided into an immunocompromised group (n = 80) and an immunocompetent group (n = 154) on the basis of their immunological status. Among the 80 immunocompromised patients, 43.75% (35/80) had hematological diseases, 28.75% (23/80) had undergone chemotherapy for solid tumors in the past 3 months, 13.75% (11/80) had a daily intake of more than 20 mg of glucocorticoids for more than 14 days (or a cumulative dose of 700 mg prednisolone or equivalent doses of other corticosteroids), and 13.75% (11/80) had an immunocompromised status due to hereditary or congenital factors (Fig. 2).

There was no statistically significant difference in age or sex between the immunocompromised group and the immunocompetent group ($P > 0.05$) (Table 1). In the immunocompromised group, the levels of platelets, hemoglobin, and white blood cells were significantly lower than those in the immunocompetent group ($P < 0.05$), while the levels of D-dimer, procalcitonin (PCT), and C-reactive protein (CRP) were significantly higher than the immunocompetent group ($P < 0.05$). The proportion and duration of mechanical ventilation were not significantly different between the two groups ($P > 0.05$), and the duration of ICU stay in the

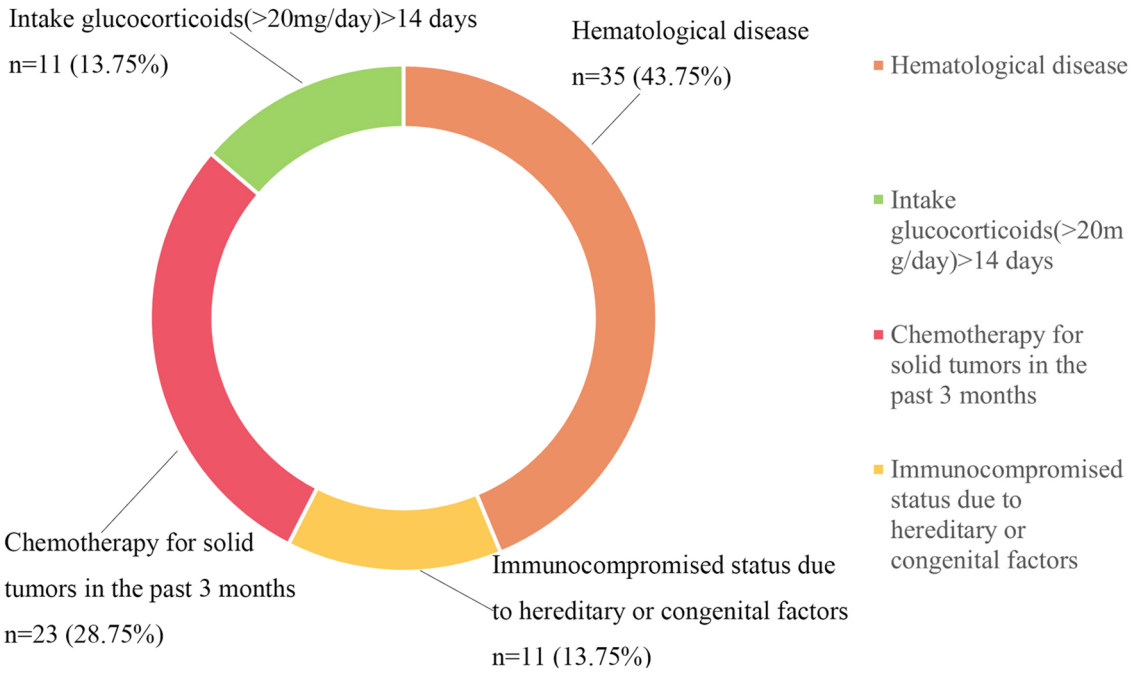


Fig. 2 The distribution of immunocompromised status in immunocompromised patients

immunocompromised group was significantly shorter than that in the immunocompetent group [8.00 (5.00–16.75) vs 12.00 (6.00–20.00), $P<0.05$]. Although there was no statistically significant difference in ICU mortality or 28-day mortality between the two groups, the APACHE II score in the immunocompromised group was significantly higher than that in the immunocompetent group [31 (27.25–35.00) vs 26 (23.00–28.00), $P<0.05$].

Comparison of the diagnostic performance of mngs to cmts in all patients

Among the 234 patients included in this study, the Kappa analysis results indicated poor consistency between mNGS and CMTs ($P=0.414$) (Table 2). Among these methods, both methods yielded positive results in 63.68% (149/234) of the cases, and both methods yielded negative results in 2.56% (6/234) of the cases. Additionally, 30.34% (71/234) of patients tested positive with only mNGS, whereas 3.42% (8/234) tested positive with only CMTs. Among the 149 patients who tested positive with both methods, only 9 patients (6.04%) were completely matched, 43 patients (28.86%) were mismatched, and 97 patients (65.10%) were partially matched (Fig. 3A). A total of 174 pathogens were detected, with mNGS identifying 167 species (95.98%) and CMTs detecting 51 species (29.31%). Among CMTs, the method with the highest pathogen detection rate was BALF culture (n=23, 13.22%), followed by sputum culture (n=22, 12.64%), blood culture (n=20, 11.49%) and PCR (n=12, 6.90%) (Fig. 3B).

Comparison of the diagnostic performance of mngs to cmts in both groups

In the immunocompromised group, the positivity rates of mNGS were significantly higher than those of CMTs (95% vs 58.75%, $P<0.05$). In the immunocompetent group, mNGS also had a higher positivity rate than CMTs did (93.51% vs 71.43%, $P<0.05$). Notably, the positivity rate of CMTs was significantly lower in the immunocompromised group than in the immunocompetent group (58.75% vs 71.43%, $P<0.05$) (Fig. 4).

In the immunocompromised group, both methods yielded positive results in 56.25% (45/80) of the patients and negative results in 2.50% (2/80) of the patients (Fig. 5A). Furthermore, 38.75% (31/80) of patients tested positive with only mNGS, whereas 2.50% (2/80) tested positive with only CMTs. Among the 45 patients who tested positive with both methods in the immunocompromised group, only 4 patients (10.26%) were completely matched, 15 patients (38.46%) were mismatched, and 26 patients (57.78%) were partially matched.

In the immunocompetent group, both methods yielded positive results in 67.53% (104/154) of the patients and negative results in 2.60% (4/154) of

Table 1 Basic clinical data and medical history of the patients

	Immunocompromised group (n = 80)	Immunocompetent group (n = 154)	*P* value
Age, years (M, IQR)	67 (59–75)	70 (58–79)	0.081
Female (n, %)	42 (52.50%)	76 (49.35%)	0.582
Fever (n, %)	46 (57.50%)	86 (55.84%)	0.809
Comorbidity			
Hypertension (n, %)	21 (26.25%)	61 (39.61%)	0.043
Diabetes (n, %)	9 (11.25%)	30 (19.48%)	0.110
Heart disease (n, %)	6 (7.50%)	17 (11.04%)	0.389
Clinical laboratory data			
WBC, $\times 10^9$/L (M, IQR)	5.80 (1.93–12.90)	9.95 (7.48–13.60)	0.000
PCT, ng/ml (M, IQR)	4.78 (0.46–37.21)	1.42 (0.46–6.82)	0.009
CRP, mg/L (M, IQR)	131.70 (60.88–213.48)	104.96 (46.60–172.55)	0.041
Platelets, $\times 10^9$/L (M, IQR)	84.00 (59.25–108.5)	114.5 (64.75–169.75)	0.000
Hemoglobin, g/L(M, IQR)	92.00 (77.00–108.25)	102.50 (81.75–117.00)	0.030
Neutrophil count, $\times 10^9$/L (M, IQR)	9.69 (5.47–15.65)	8.90 (5.93–12.19)	0.500
Neutrophil%, (M, IQR)	89.90 (79.65–92.48)	86.55 (79.03–92.33)	0.257
Bilirubin, μmol/L (M, IQR)	12.95 (8.23–20.00)	12.85 (8.28–20.83)	0.912
Creatinine, μmol/L (M, IQR)	83.10 (56.18–142.98)	95.50 (58.43–186.15)	0.363
Lymphocyte%, (M, IQR)	0.59 (0.29–0.79)	0.62 (0.38–0.79)	0.154
D-dimer, mg/L (M, IQR)	4.04 (2.06–9.36)	2.70 (1.48–7.18)	0.047
APACHE-II score	31 (27.25–35.00)	26 (23.00–28.00)	0.000
ICU treatment			
MV (n, %)	65 (81.25%)	133 (86.36%)	0.305
Duration of MV (day)	7.00 (2.00–14.75)	9.00 (3.00–17.25)	0.139
ECMO treatment (n, %)	3 (3.75%)	6 (3.90%)	0.956
Length of ICU stay (day)	8.00 (5.00–16.75)	12.00 (6.00–20.00)	0.035
ICU Mortality (n, %)	14 (17.50%)	27 (17.53%)	0.995
28-day mortality (n, %)	34 (42.50%)	58 (37.66%)	0.473

WBC white blood count, *PCT* procalcitonin, *CRP* C-reactive protein, *APACHE-II score* Acute Physiology and Chronic Health Evaluation II score, *MV* mechanical ventilation, *ECMO* extracorporeal membrane oxygenation

Table 2 Kappa analysis of concordance between mNGS and CMTs results

mNGS	CMTs		Total
	Positive (+)	Negative (−)	
Positive (+)	149	71	220
Negative (−)	8	6	14
Total	157	77	234

P = 0.414, Kappa value = 0.034

the patients (Fig. 5B). A total of 25.97% (40/154) of patients tested positive with only mNGS, and 3.90% (6/154) tested positive with only CMTs. Among the 104 patients who tested positive with both methods in the immunocompetent group, only 5 patients (4.81%) were completely matched, 27 patients (26.92%) were mismatched, and 71 patients (68.27%) were partially matched.

Differences between cmts and mngs in detecting pathogenic microorganisms

mNGS had a significantly greater detection rate for pathogenic microorganisms than did CMTs (94.02% vs 66.67%, $P < 0.05$). The detection rates for bacteria (86.32% vs 57.26%, $P < 0.05$) and fungi (44.87% vs 23.93%, $P < 0.05$) were notably greater than those for CMTs. Additionally, mNGS demonstrated excellent diagnostic performance in detecting viruses (44.02% vs 11.54%, $P < 0.05$) compared with CMTs (Fig. 6).

Among the microbes identified by both methods in all patients (Fig. 7), the most frequently identified bacteria were *Klebsiella pneumoniae* (n = 93), *Acinetobacter baumannii* (n = 89), *Stenotrophomonas maltophilia* (n = 69), *Corynebacterium striatum* (n = 56), *Enterococcus faecium* (n = 49), *Staphylococcus aureus* (n = 42), *Pseudomonas aeruginosa* (n = 38),

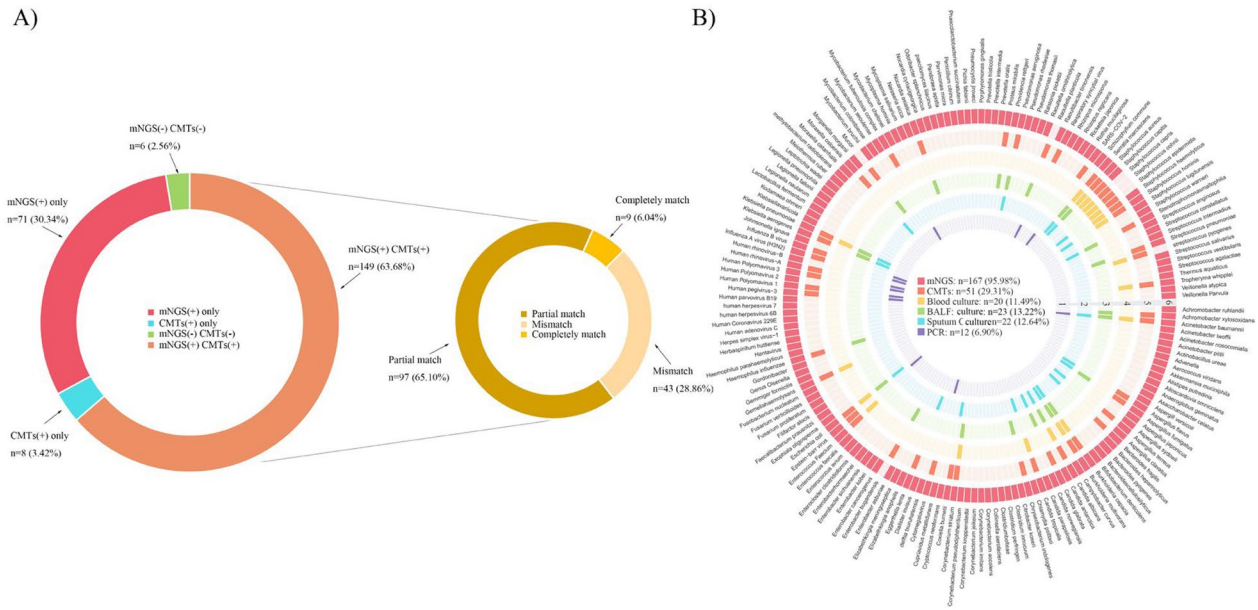


Fig. 3 **A** Pathogen identification consistency between mNGS and CMTs. **B** Pathogen species detection by mNGS or CMTs

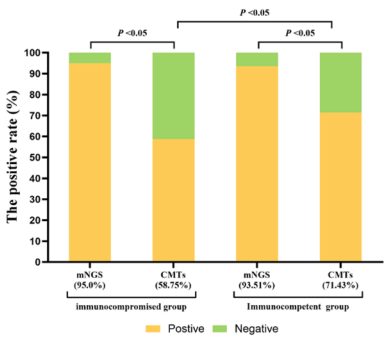


Fig. 4 Comparison of the positivity rates between mNGS and CMTs

and *Serratia marcescens* (n = 27). Among the fungal organisms detected, *Candida albicans* (n = 93) was the most common, followed by *Aspergillus fumigatus* (n = 30) and *Candida glabrata* (n = 22). Among the viruses detected, the most common were Herpes simplex virus-1 (n = 30), Human herpesvirus 7 (n = 14), and Human herpesvirus 6B (n = 4).

A total of 174 pathogens were detected via mNGS and CMTs, and 123 and 7 pathogens were detected only by mNGS or CMTs, respectively. The 123 pathogens identified by mNGS included 95 bacteria, 19 fungi, and 9 viruses. Among these 123 pathogens, the most common are Herpes simplex virus-1, Human herpesvirus, and *Elizabethkingia anophelis*. The 7 pathogens identified by CMTs included 6 bacteria and 1 fungus: *Enterobacter bugandensis*, *Raoultella planticola*, *Staphylococcus capitis*, *Staphylococcus cohnii*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Mucor*.

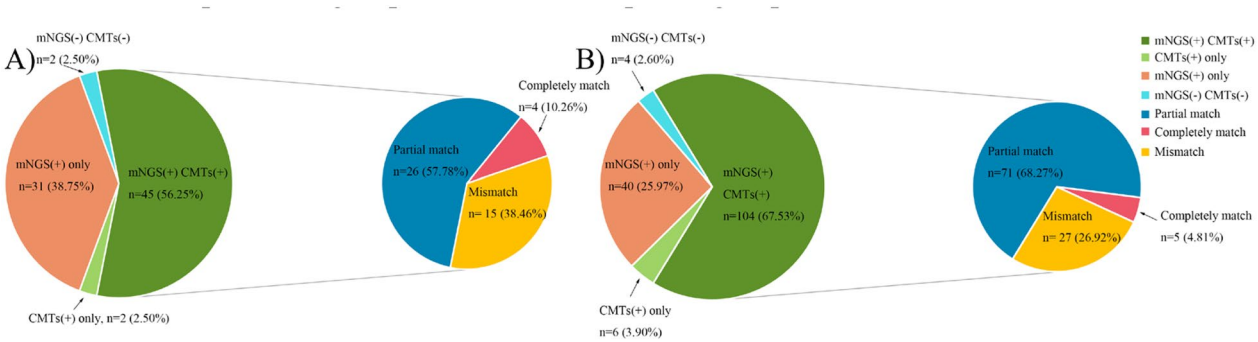
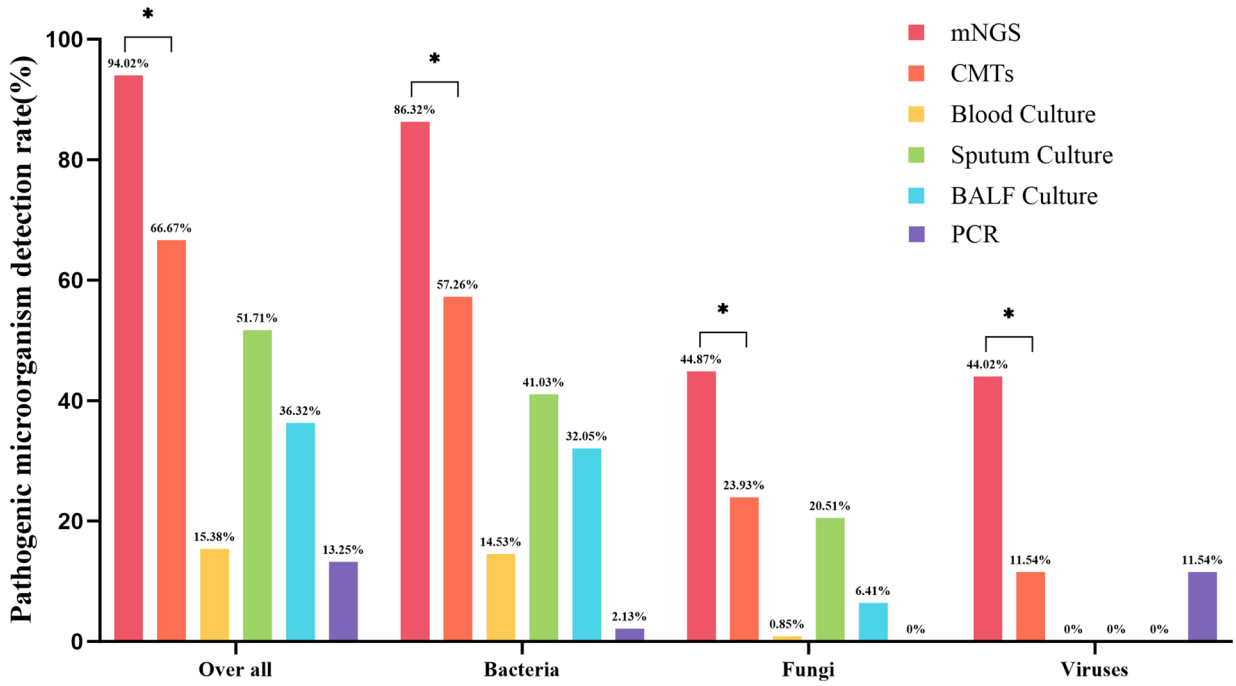


Fig. 5 Pathogen identification consistency between mNGS and CMTs. **A** Immunocompromised group. **B** Immunocompetent group



*$P<0.05$

Fig. 6 Comparing the detection rates of pathogenic microorganisms using different methods

Clinical impact of mngs or cmts results

The rates of antibiotic regimen adjustments in the immunocompromised group and immunocompetent group were 87.5% and 64.29%. Antibiotic regimen adjustments guided by mNGS were significantly more common in the immunocompromised group (70.00% vs 17.50%, $P<0.05$) and the immunocompetent group (48.70% vs 15.58%, $P<0.05$) than in the group guided by CMTs. Antibiotic escalation guided by mNGS was more common in both the immunocompromised group (28.75% vs 2.50%, $P<0.05$) and the immunocompetent group (25.32% vs 7.79%, $P<0.05$) than in the CMTs group.

In the immunocompromised group, adjunctive antifungal and antiviral treatments guided by mNGS were used in 15.00% and 8.75% of the patients, no patients received additional antifungal or antiviral treatment guided by CMTs. In the immunocompetent group, adjunctive antifungal and antiviral treatments guided by mNGS were used in 11.04% and 4.55% of the patients, respectively, 3.25% of the patients received adjunctive antifungal treatment guided by CMTs, no patients received antiviral treatment guided by CMTs.

In the immunocompromised group, both ICU mortality and 28-day mortality were significantly lower with mNGS guidance than with CMTs guidance (8.93% vs 50.00%, $P<0.05$; 30.36% vs 68.75%, $P<0.05$). However, in the immunocompetent group, no statistically significant differences were observed when comparing ICU mortality and 28-day mortality between mNGS and CMTs guidance (20.00% vs 33.33%, $P>0.05$; 42.67% vs 45.83%, $P>0.05$) (Table 3).

Further analysis showed that mNGS results had a positive effect on 68 patients (85.0%) and no effects on 10 patients (12.5%), while 2 patients (2.5%) experienced negative clinical effects. Among the 68 patients with positive effects, mNGS results provided definitive diagnoses. For the two patients with negative effects, the mNGS results led to incorrect diagnoses, ultimately resulting in inappropriate antibiotic treatment. In the group of 10 patients with no clinical effect, 4 patients failed to detect any pathogens. Additionally, the results for 6 patients were identified as either non-pathogenic organisms or normal respiratory flora/colonizers. (Table 4).

Discussion

Our study compared mNGS and CMTs techniques for detecting pathogens in immunocompromised patients with SRIs in the ICU. The results showed that mNGS has superior detection capabilities, accurately identifying pathogens even when CMTs results are negative. It demonstrated that mNGS can provide crucial support for the targeted selection of antibiotics for

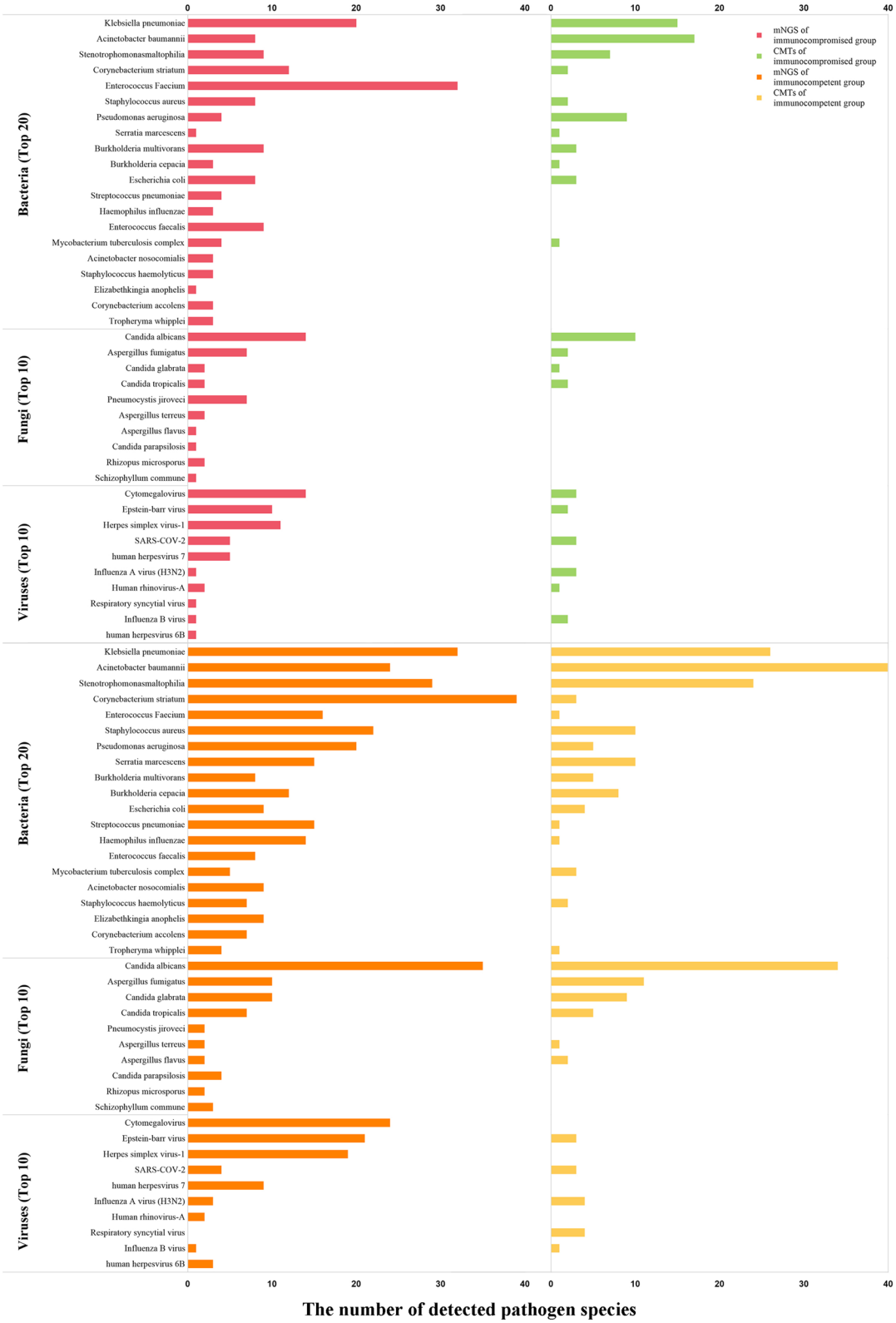


Fig. 7 The distribution of detected pathogen species by mNGS or CMTs in all patients

Table 3 The impact of antibiotic regimen adjustments and patient prognosis based on mNGS and CMT results

	Immunocompromised group (n = 80)		Immunocompetent group (n = 154)	
	mNGS	CMTs	mNGS	CMTs
Antibiotic regimen adjustments	56 (70.00%)*	14 (17.50%)	75 (48.70%)*	24 (15.58%)
Antibiotic changed	13 (16.25%)	12 (15.00%)	22 (14.29%)*	3 (1.95%)
Antibiotic escalated	23 (28.75%)*	2 (2.50%)	39 (25.32%)*	12 (7.79%)
Antibiotic de-escalated	4 (5.00%)		6 (3.90%)	1 (0.65%)
Adjunctive antifungal	12 (15.00%)		17 (11.04%)	5 (3.25%)
Adjunctive antiviral	7 (8.75%)		7 (4.55%)	
Adjunctive anti-tuberculosis	1 (1.25%)		1 (0.65%)	2 (1.30%)
ICU Mortality	5 (8.93%)*	7 (50.00%)	15 (20.00%)	8 (33.33%)
28-day mortality	17 (30.36%)*	11 (68.75%)	32 (42.67%)	11 (45.83%)

Comparison the antibiotic regimen adjustments and patient prognosis between guided by mNGS and CMTs in immunocompromised group or immunocompetent group. *$P < 0.05$

Table 4 The Clinical effect of mNGS result in immunocompromised group

Clinical effect	Role of mNGS result	Treatment changes owing to mNGS
Positive effect (n = 68, 85.0%)	Contributed to definitive diagnosis (n = 68, 85.00%)	Antibiotic treatment adjustment (n = 56; 70.0%)
		Empirical treatment continued (n = 12; 15.0%)
Negative effect (n = 2, 2.5%)	mNGS results led to incorrect diagnoses (n = 6, 7.5%)	Incorrect antibiotic treatment
No effect (n = 10, 12.5%)	Negative mNGS results (n = 4, 5.0%)	No changes
	Results deemed false or insignificant (n = 6, 7.5%)	No changes

immunocompromised patients and could significantly improve their prognosis.

Owing to the significant increase in effective interventions for cancer, organ transplants, hematologic diseases, and autoimmune disorders, the proportion of immunocompromised patients in the ICU is steadily increasing [25]. These patients are at increased risk of developing severe respiratory infections due to immune abnormalities, the use of broad-spectrum antibiotics during ICU stays, and the use of additional immunotherapies. Which can lead to multiorgan dysfunction, septic shock, and ARF, all of which are major causes of mortality in these patients.

The main diseases associated with severe respiratory infections in immunocompromised patients in the ICU are community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), ventilator-associated lower respiratory infections (VA-LRTI) [26]. Notably, these patients appear to be more susceptible to infections by less virulent bacteria, as well as fungi and viruses, than immunocompetent patients are, leading to "opportunistic infections" [27]. Additionally, these patients have a greater incidence of multidrug-resistant organisms.

Invasive fungal infections are a major cause of morbidity and mortality among immunocompromised individuals [2, 28]. These patients are prone to opportunistic fungal infections, such as *Aspergillus* and *Pneumocystis jirovecii*, because of their exposure to the ICU environment [29]. However, fungal cultures are time-consuming and labor-intensive, requiring pure isolates with spore formation and distinctive characteristics for identification through macro- and microscopic morphology [30]. CMTs often fail to provide timely etiological information, posing challenges for antifungal treatment in immunocompromised patients and leading to poor prognosis. A previous study indicated that mNGS can quickly provide etiological insights and facilitate early antifungal treatment, effectively reducing infection recurrence rates and improving patient prognosis [31]. Owing to the reliance on advanced cell wall disruption techniques and the relatively low fungal content in BALF, fungal identification through mNGS still faces challenges. Shi et al. [32] reported that mNGS has significant value in detecting fungal pathogens in patients with negative CMTs results. In this study [32], the sensitivity, specificity, and accuracy of mNGS for fungal detection were 82.6%, 97.7%, and 92.5%, respectively. Notably, the sensitivity of mNGS surpassed that of any single CMTs. In our study, mNGS also demonstrated excellent identification value for fungi, the detection rate was significantly higher than that of CMTs (44.87% vs 23.93%, $P < 0.05$).

mNGS significantly outperformed CMTs in detecting *Candida* spp., *Aspergillus* spp., and *Pneumocystis jirovecii*. Notably, CMTs had a detection rate of 0% for *Pneumocystis jirovecii*, whereas mNGS revealed 3 cases in the immunocompromised group and timely adjustments in antifungal treatment on the basis of the findings. These results indicate that mNGS can significantly increase the detection rate of fungi, providing clinicians with more references for accurate diagnosis and treatment of fungal infections in immunocompromised patients.

Viral infections also play a crucial role in the incidence of respiratory infections in immunocompromised individuals in the ICU [33, 34]. ICU-acquired viral infections in this population may result from nosocomial acquisition or reactivation of latent viruses [2]. A previous study demonstrated that the detection rate of rhinoviruses/enteroviruses is gradually increasing among patients with hematologic diseases, reaching 56% [35]. Parainfluenza virus [36] and Respiratory syncytial virus [37] are more common in hematopoietic stem cell transplant patients. Reactivation of Cytomegalovirus is common in patients undergoing invasive mechanical ventilation [38], and other herpes viruses, such as Epstein–Barr virus and Herpes simplex virus, can remain latent in the body for long periods after initial infection and reactivate under conditions of immunosuppression. Therefore, assessing the clinical significance of detected herpes viruses based on viral load, clinical symptoms and radiological examination to adjust antiviral treatments accordingly is crucial for improving patient outcomes. The detection of viral infections in immunocompromised patients currently relies primarily on PCR. However, owing to its reliance on target sequence information, and the dependence on physician judgment to select which viruses to test, PCR testing faces challenges in clinical application. Studies have shown that in cases where pathogens and diagnoses are unclear, the detection rate of viruses via PCR is only 0.9%, whereas mNGS achieves a detection rate of 43.8% [39]. In our study, the detection rate of viruses via mNGS was significantly superior to that via CMTs (44.02% vs 11.54%, $P<0.05$), which is consistent with the findings of previous studies. The most frequently detected viruses were Cytomegalovirus, Epstein–Barr virus, and Herpes simplex virus 1, which is in agreement with past research findings [40]. These viruses are not routinely screened in immunocompromised patients with severe respiratory infections in China. In our study, 7 patients in the immunocompromised group had their antiviral treatment adjusted based on mNGS results, whereas no patients had their antiviral treatment adjusted based on CMTs results. The application of mNGS provided a clear diagnosis for antiviral treatment and significantly improved patient prognosis.

The application of mNGS significantly increased the detection rate of pathogenic microorganisms, providing important references for clinicians' diagnosis and treatment. CMTs often struggle to identify rare pathogens, but mNGS can fill this gap. In this study, mNGS detected 13 cases of *Mycobacterium tuberculosis*, 7 cases of *Pneumocystis jirovecii*, 2 cases of *Nocardia*, 2 cases of *Fusarium*, and 1 case of *Dialister invisus*. These pathogens are rare but critically important opportunistic pathogens in immunocompromised patients. When conventional methods face obstacles such as low genetic load, antibiotic exposure, or inherent limitations, clinicians can use mNGS to identify the types of pathogens causing poor response to standard antibiotic regimens [41], particularly in immunocompromised patients who are prone to complex infections and rare pathogen infections.

In this study, mNGS showed a significant lack of consistency in pathogen detection rates compared with CMTs (Kappa value = 0.034, $P=0.414$). Although 63.68% (149/234) of patients had double-positive results, among these patients, only 9 (6.04%) were completely matched, 43 (28.86%) were mismatched, and 97 (65.10%) were partially matched. Similar results were observed in both the immunocompromised group and the immunocompetent group. The reason for this situation is that mNGS is often able to detect a greater number of pathogens. However, clinicians should be cautious when interpreting mNGS data, distinguishing between normal colonizing bacteria and pathogens causing infection, to develop the most appropriate antibiotic treatment regimen [9, 23].

mNGS can identify pathogens and guide clinicians in adjusting antibiotic treatment regimens. In our study, the percentages of antibiotic treatment adjustments guided by mNGS in the immunocompromised and immunocompetent groups were 70% and 48.70%, respectively. In the immunocompromised group, the ICU and 28-day mortality rates were significantly lower in patients whose antibiotic regimens were adjusted on the basis of mNGS results than CMTs. However, in the immunocompetent group, these results did not demonstrate statistical significance. Similar findings were reported by Zhao et al. in 2022 [42], showed mNGS also play a relatively important role in detecting mixed pathogens and personalized antibiotic treatment in immunocompromised patients. Notably, in our study, the average length of hospital stay was significantly shorter in the immunocompromised group than in the immunocompetent group. mNGS plays a positive role for immunocompromised patients in identifying mixed infections, assisting in diagnosis, adjusting antibiotic treatment regimens, and reducing the length of hospital stay.

This study comprehensively compared the diagnostic efficiency of conventional methods and mNGS for ICU immunocompromised patients with SRIs and evaluated the clinical impact of mNGS on these patients; however, it also has several limitations. First, the limited sample size in this study may affect the accuracy of mNGS performance evaluation. Second, mNGS was performed only on BALF samples, while other types of samples, such as blood, sputum, and cerebrospinal fluid, were not included. Additionally, CMTs were limited to blood culture, BALF culture, sputum culture, and PCR testing, without incorporating other methods such as GM tests, G tests, and serological assays. Finally, the interpretation of mNGS results relies on clinicians' judgment of colonizing versus pathogenic microbes. To further assess the application of mNGS in the diagnosis of SRIs in immunocompromised patients in the ICU, future research with larger sample sizes and multicenter prospective studies is needed.

Abbreviations

ICU	Intensive care unit
APACHE-II score	Acute physiology and chronic health evaluation II score
ARF	Acute respiratory failure
BALF	Bronchoalveolar lavage fluid
CAP	Community-acquired pneumonia
CMTs	Conventional microbiological tests
CRP	C-reactive protein
CT	Chest computed tomography
ECMO	Extracorporeal membrane oxygenation
HAP	Hospital-acquired pneumonia
IQR	Interquartile ranges
mNGS	Metagenomic next-generation sequencing
MV	Mechanical ventilation
PCR	Polymerase chain reaction
PCT	Procalcitonin
SRIs	Severe respiratory infections
VA-LRTI	Ventilator-associated lower respiratory infections
WBC	White blood count

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Author contributions
H.F. and J.Z. conceptualized the study. J.Z. developed the methodology. J.Z. was responsible for the software. J.Z. conducted the formal analysis. Resources were provided by H.F. and Y.S. Data curation was handled by Y.S., J.T., K.G., and K.W. J.Z. wrote the original draft. Supervision was provided by H.F. and J.ZG. All authors read and reviewed the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The retrospective studies involving humans were approved by the ethics committee of Quzhou People's Hospital (Quzhou People's Hospital, Quzhou, China: Number B 2024-092). The studies were conducted in accordance with local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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