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Application Value of Metagenomic Next-Generation Sequencing Using Bronchoalveolar Lavage Fluid and Blood Samples in Patients with Severe Pneumonia Complicated with Bloodstream Infection

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Abstract

This study was designed to systematically evaluate the diagnostic performance of metagenomic next-generation sequencing (mNGS) using blood and bronchoalveolar lavage fluid (BALF) samples in patients with severe pneumonia complicated by bloodstream infections. A retrospective analysis of 30 patients with severe pneumonia-bloodstream infection admitted to our hospital from January 2018 to December 2022 was conducted, and the potential pathogens in both BALF and blood samples were simultaneously detected by conventional microbial examination (traditional group) and mNGS tests (mNGS group), comparing the differences in pathogen species and detection rates between the two methods. There was no significant difference in the positivity of pathogen detection in BALF and blood samples using mNGS ($p = 0.492$). The proportion of bacteria ($p = 0.005$) and fungi ($p = 0.037$) detected by BALF mNGS was higher than that by blood mNGS, but there was no significant difference in the proportion of viruses ($p = 0.121$). In addition, the positive rate of pathogen detection by mNGS in BALF and blood samples was significantly higher than that by traditional methods ($p < 0.01$). BALF mNGS demonstrated superior diagnostic sensitivity for bacterial and fungal pathogen detection compared to blood mNGS and conventional culture methods. Notably, blood specimens retained distinct advantages in identifying specific viral infections. Future prospective studies with larger sample sizes are warranted to validate these findings.

Keywords: severe pneumonia, bloodstream infection, pathogen, metagenomic next-generation sequencing, viruses

Introduction

Severe pneumonia is an acute and critically ill respiratory disease with a high global mortality rate and is often accompanied by severe inflammatory reactions (Chiu and Miller 2019). Bloodstream infection is one of its common complications. Bloodstream infection refers to the presence of pathogenic microorganisms in the patients' blood, with signs and symptoms of infection, and is a systemic infection that endangers human life. Pathogenic microorganisms that cause bloodstream infections include bacteria, fungi and viruses, which can lead to bacteremia, septicemia and sepsis, and in severe cases, shock, disseminated intravascular coagulation (DIC), multiple organ failure and even death (Shi et al. 2019). Severe inflammatory stress reactions occur in patients with severe pneumonia complicated with bloodstream infection, leading to the release of a large number of inflammatory factors. However, clinical inflammatory diagnostic indi-

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cators are numerous, and it is difficult to select clinical characteristic indicators to judge the progression and prognosis of severe pneumonia (Evans et al. 2021). Anti-infection is the core content of severe pneumonia, and the pathogen is difficult to diagnose due to a wide variety of pathogens, so identifying the pathogen is the key (Walden et al. 2014; Chen et al. 2020). Therefore, innovative detection techniques are urgently needed to improve the diagnostic efficiency of severe pneumonia complicated with bloodstream infection and to accurately formulate treatment strategies.

The technology of metagenomic next-generation sequencing (mNGS) requires no culture and presumptions to directly conduct high-throughput sequencing of nucleic acid substances in samples to obtain huge sequence data, and analyze pathogen and abundance information in samples through microbial sequence database comparison (Cillóniz et al. 2021). Through metagenomic sequencing of clinical specimens, mNGS can detect a variety of microorganisms (including viruses, bacteria, fungi and parasites) in specimens without bias. Currently, mNGS has been widely used in pathogen detection of clinical infectious diseases (Langelier et al. 2018), which covers a wider range and is unbiased compared with traditional clinical microbial pathogen detection (Li et al. 2018). More and more clinical studies and reports of infection cases of special pathogens, especially rare caustic pathogens, confirm the important value of mNGS in the diagnosis of infectious disease pathogens (Lanks et al. 2019; Yang et al. 2022). However, interpretation of mNGS results must consider the clinical context, as pathogens detected in non-sterile sites like bronchoalveolar lavage fluid (BALF) may represent colonization rather than active infection. For MDR organisms, genotypic resistance profiles from mNGS data should be validated through clinical follow-up to ensure appropriate empirical antibiotic selection and minimize resistance development. In this study, we analyzed the efficacy of mNGS in patients with severe pneumonia complicated with bloodstream infection, discussed and analyzed the clinical utility value of mNGS (plasma mNGS and blood mNGS) in detection and identification of pathogens of severe pneumonia complicated with bloodstream infection. Furthermore, the diagnostic performance of mNGS using BALF and blood samples in patients with severe pneumonia complicated with bloodstream infection was evaluated by comparing with conventional diagnostic methods.

Experimental

Materials and Methods

Patient subjects. This retrospective study included 30 patients with severe pneumonia complicated by bloodstream infection admitted to our hospital between January 2018 and December 2022. This research was approved by the ethics committee of First People's Hospital of Nanning Hospital. All of these patients are hospitalized in the respiratory intensive care unit (ICU). All patients signed the informed consent. The following inclusion criteria were used: According to the diagnostic criteria for severe pneumonia established by the Infectious Diseases Society of America (IDSA)/American Thoracic Society (ATS), the main criteria included: (1) the need for invasive mechanical ventilation; (2) septic shock requiring pressor drugs. Secondary criteria included: (1) respiratory rate greater than 30 beats/min; (2) oxygenation index <250 mmHg; (3) multiple lung lobes involved; (4) disturbance of consciousness and/or disorientation; (5) uremia, BUN > 20 mg/dl; (6) hypotension (systolic blood pressure <90 mmHg) requires fluid resuscitation. Severe pneumonia can be diagnosed if one of the main criteria or at least three secondary criteria are met. The diagnostic criteria for bloodstream infections in this study were comprehensively determined based on clinical manifestations, hematological laboratory tests, and plasma metagenomic analysis, as follows: (1) Clinical features: Presence of systemic infection symptoms including abnormal body temperature (fever >38°C or hypothermia <36°C), accompanied by chills, tachycardia, tachypnea, hypotension, or cutaneous petechiae. Critical cases may progress to septic shock or multiple organ dysfunction syndrome (MODS). (2) Hematological laboratory parameters: Abnormal peripheral white blood cell count ($>12 \times 10^9$/l or $<4 \times 10^9$/l), or immature neutrophil proportion exceeding 10%, combined with C-reactive protein (CRP) concentration >50 mg/l or procalcitonin (PCT) level >0.5 ng/ml. (3) Positive plasma metagenomic testing results.

Exclusion criteria: (1) individuals with HIV-induced immunodeficiency; (2) those who gave up treatment or withdrew from treatment for other reasons.

Samples and laboratory testing. Patients with severe pneumonia with bloodstream infection were divided into two groups, the mNGS group and the traditional detection group. After the patient was ad-

mitted to the ICU, ECG monitoring was performed routinely, and appropriate respiratory support, direct arterial pressure measurement, and central venous catheterization were administered according to the patient's clinical condition. Empirical antibiotic therapy was started within 1h after admission. BALF and blood samples were obtained in all patients within 24 h after admission. All BALF procedures were performed within the ICU setting, and all enrolled patients were receiving invasive positive pressure ventilation (IPPV) via artificial airways established through either endotracheal intubation or tracheostomy. Each specimen was equally divided into two parts for mNGS analysis and traditional pathogen detection. The mNGS group underwent comprehensive pathogen profiling using high-throughput sequencing technology for both BALF and blood specimens. In contrast, the conventional detection group underwent multi-modal diagnostic evaluation comprising: (1) microbial cultures (quantitative BALF culture, blood culture); (2) smear; (3) PCR of blood and throat swabs (including Parvovirus B19, Herpes Simplex virus 1/2, Epstein-Barr virus, Cytomegalovirus, SARS-CoV-2, respiratory syncytial virus, influenza A/B, parainfluenza viruses 1–4, adenovirus, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*); and (4) serological assays (1,3-β-D-glucan assay, galactomannan test, and *M. pneumoniae*-specific IgM/IgG antibody detection).

The mNGS and bioinformatics analyses. BALF and blood samples were obtained following standard aseptic procedures. The samples were immediately transported to a genetic testing company (Changsha Kingmed Medical Test Center Co. Ltd., China) under cold-chain conditions. The genetic testing was carried out according to the previous description. Briefly, genomic DNA was isolated from 1 mL bronchoalveolar lavage fluid (BALF) specimens using the QIAamp® UCP Pathogen DNA Kit (Qiagen, Germany) following the manufacturer's protocol. To deplete host-derived DNA, samples were treated with Benzonase® nuclease (Qiagen, Germany) in conjunction with Tween® 20 detergent (Sigma-Aldrich, USA). Total RNA extraction was subsequently performed with the QIAamp® Viral RNA Kit (Qiagen, Germany), followed by ribosomal RNA depletion employing the Ribo-Zero™ rRNA Removal Kit (Illumina, USA). Paired DNA and cDNA libraries were then constructed using the QIAseq® Ultralow Input Library Kit for Illumina® sequencing platforms (Qiagen, Germany). To obtain the clean data, the adapter sequences, low-quality data, and polyG tails were removed by FastQC software. The sequences that could be mapped to the human reference genome (human reference build GRCh38) were then filtered out using Burrow-Wheeler Aligner. The pathogenic microorganism database of Guangzhou Sagene Biology, which contained information on bacterial, viral, fungal, and parasitic species, was used to align the remaining microbial data. References from the NCBI database, the Ensemble database, the Virus Resource database, the JGI Fungi Porta, and other authoritative microorganism databases were acquired by this database. mNGS data were analyzed using standardized specifically mapped read numbers (SDSMRN), calculated by normalizing the specifically mapped read count of each microbial taxon to 20 million total sequencing reads. Pathogen identification criteria were defined as follows: (1) Bacterial, mycoplasmal, chlamydial, DNA viral, and fungal pathogens: SDSMRN ≥ 3; (2) Parasitic pathogens: SDSMRN ≥ 100; (3) *Mycobacterium tuberculosis* complex (MTC): SDSMRN ≥ 1.

Statistical analysis. Statistical analyses were conducted using SPSS 25.0 software (SPSS Inc., USA). The Kolmogorov-Smirnov test was applied to assess the normality of data distribution for each variable. Quantitative variables were described using mean ± standard deviation (SD) when following a normal distribution, and median [interquartile range] for non-normally distributed data. Categorical variables were presented as absolute frequencies (n) and relative percentages (%). Between-group comparisons for continuous variables were performed using Student's t-test for normally distributed data or the Mann-Whitney U test for non-parametric distributions. Categorical data were analyzed with Pearson's chi-square test. Statistical significance was defined as $p < 0.05$ (two-tailed).

Results

Demographic data analysis. The current study enrolled 30 patients with severe pneumonia complicated with bloodstream infection, of whom 21 were males and 9 were females. The patients' median age was 66.5 ± 15.1 years (Table I). None of the patients had a history of hematological diseases, including leukemia or transplantation. The ventilator use time was 12.17 ± 17.32 days, the average ICU hospitalization time was 18.27 ± 20.1 days. Additionally, 1 (3.3%) of the patients

Table I
Baseline data characteristics of patients with severe pneumonia complicated with bloodstream infection.

Patient characteristics	All patients (n = 30)
Age, years	66.5±15.1
Gender, n (%)	
Male	21 (70%)
Female	9 (30%)
Comorbidity	
Hypertension, n (%)	17 (56.7%)
Heart disease, n (%)	1 (3.3%)
Diabetes mellitus, n (%)	5 (16.7%)
Cerebral infarction, n (%)	5 (16.7%)
Solid tumors, n (%)	2 (6.67%)
Alcoholic hepatitis, n (%)	2 (6.67%)
Smoking, n (%)	6 (20%)
APACHE II score	26.2±6.76
SOFA score	10.47±4.65
Ventilator use time (days)	12.17±17.32
ICU time (days)	18.27±20.1
Prior hormones/immunosuppressants exposure, n (%)	1 (3.3%)

had been treated with hormones/immunosuppressants before sampling. The mean APACHE II score was 26.2 ± 6.76, and the mean SOFA score was 10.47 ± 4.65.

Pathogen detection by mNGS in BALF and blood samples. The results showed that out of the 30 patients included in the study, all of them (100%) tested positive for pathogens when using mNGS on BALF samples. Similarly, in the blood samples, 28 patients (93.3%) tested positive for pathogens using mNGS. The comparison of pathogen positivity between BALF and blood samples did not yield a significant difference ($p = 0.492$). Among the BALF samples, 5 cases (16.7%) showed a single pathogen, while 25 cases (83.3%) had multiple pathogens. In contrast, among the blood samples, 7 cases (25%) had a single pathogen, and 21 cases (75%) had multiple pathogens. The total number of pathogens detected in BALF and blood samples using mNGS was 37 and 23, respectively. In terms of the specific pathogens detected, mNGS identified a total of 37 pathogens in BALF samples, including 25 bacteria (including 9 Gram-positive bacteria and 16 Gram-negative bacteria), 6 viruses, 5 fungi, and 1 specific pathogen. In the blood samples, mNGS detected a total of 23 pathogens, including 13 bacteria (including 6 Gram-positive bacteria and 7 Gram-negative bacteria), 7 viruses, and 3 fungi. The most frequently detected pathogens using BALF mNGS were *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Aspergillus fumigatus* (Table II). Meanwhile, *K. pneumoniae*, Epstein-Barr virus, and CytoMegalo virus were the most common pathogens observed in blood mNGS results (Table II). Besides, the proportion of bacteria (93.3% *vs.* 63.3%, $p = 0.005$) and fungus (36.7% *vs.* 13.3%, $p = 0.037$) detected using BALF mNGS was higher than that using blood mNGS. However, there was no significant difference in the proportion of viruses between the positive blood mNGS results and BALF mNGS results (40% *vs.* 60%, $p = 0.121$; Table II).

Traditional microbial examination versus mNGS for bacterial detection in BALF and blood. Among the 30 patients analyzed, 20 cases (66.7%) demonstrated complete concordance between pathogens identified by BALF mNGS and those detected through BALF culture. Notably, three patients exhibited full agreement between mNGS results from both blood and BALF samples and their corresponding culture outcomes (BALF and blood cultures), with the number of pathogen-derived sequencing reads in blood mNGS consistently lower than that observed in BALF mNGS (Table SI).

The traditional microbial examination of BALF samples from 30 patients yielded positive bacterial results in 21 patients (positivity rate: 70%), demonstrating a statistically significant difference from the mNGS group (positivity rate: 100%) ($p = 0.004$). Of the 21 patients with positive traditional microbial examination, 18 (85,7%) cases were found to have a single pathogen, and 3 (14.3%) had a mix of pathogens. Of the 21 patients with positive traditional microbial examination, one patient had *Candida glabrata* detected on culture yet no bacteria were detected by mNGS, one patient had a culture result of *Candida tropicalis* yet no bacteria were detected by mNGS, and the remaining 19 patients had the same pathogenic bacteria detected by mNGS (Table III).

Additionally, we conducted a comparison between the outcomes of traditional microbial examination and mNGS of blood. The traditional microbial examination of blood samples from 30 patients yielded positive pathogen results in 3 patients (positivity rate: 10%). However, 30 patients tested positive for pathogen using mNGS of blood samples (positivity rate: 93.3%), suggesting that mNGS had a superior positive detection rate in comparison to traditional microbial examination (Table III).

Table II
A comparative detection of pathogens by mNGS from BALF and blood samples in patients with severe pneumonia complicated with bloodstream infection.

Pathogenic species	**BALF-mNGS**	**Blood-mNGS**	***p*-value**
Pathogens	30	28	0.492
Bacteria	28	19	0.005
Gram-positive bacteria	10	6	
Corynebacterium striatum	4	0	
Corynebacterium diphtheriae	1	0	
Streptococcus pneumoniae	3	2	
Streptococcus miller	1	0	
Streptococcus anginosus	1	0	
Streptococcus constellatus	2	1	
Parvimonas micra	1	0	
Enterococcus faecium	1	1	
Staphylococcus warneri	0	1	
Staphylococcus epidermidis	0	1	
Staphylococcus aureus	3	1	
Gram-negative bacteria	26	16	
Stenotrophomonas maltophilia	8	1	
Prevotella loescheii	1	0	
Prevotella intermedia	1	0	
Fusobacterium nucleatum	1	0	
Veillonella parvula	1	0	
Klebsiella pneumoniae	19	12	
Escherichia coli	5	2	
Pseudomonas aeruginosa	4	2	
Enterobacter cloacae complex	3	0	
Haemophilus influenzae	2	0	
Moraxella catarrhalis	2	0	
Acinetobacter baumannii	11	2	
Elizabethkingia anophelis	2	0	
Klebsiella quasipneumoniae	1	0	
Helicobacter pylori	0	1	
Burkholderia multivorans	1	0	
Mycoplasma, chlamydia, or Chlamydophila	1	0	1
Mycobacterium tuberculosis complex	1	0	
Fungus	11	4	0.037
Candida tropicalis	0	1	
Candida albicans	2	0	

Table III
A comparative detection of pathogens by mNGS and traditional microbial examination from BALF and blood samples.

Pathogen	BALF-mNGS	Blood-mNGS	BALF-traditional	Blood-traditional
All cases	30 (100%)	28 (93.3%)	21 (70%)	3 (10%)
Bacterial	28 (93.3%)	19 (63.3%)	19 (63.3%)	3 (10%)
Fungi	11 (36.67%)	4 (13.3%)	4 (13.3%)	0 (0%)
Virus	12 (40%)	18 (60%)	0 (0%)	0 (0%)

Discussion

Severe pneumonia triggers systemic inflammation through pulmonary vasodilation and hyperperfusion, facilitating pathogen translocation from respiratory epithelium to bloodstream and initiating cascading inflammatory responses that contribute to sepsis progression, with six-month ICU mortality reaching 27% (Walden et al. 2014). Invasive interventions exacerbate infection risks while delayed pathogen identification leads to antibiotic overuse, prolonged hospitalization, and escalated costs (Chen et al. 2020). The mNGS addresses these challenges through culture-independent pathogen detection, offering five key advantages: accelerated diagnostics (1–2 days *vs.* 3–5 days for cultures), broad-spectrum identification of bacteria/fungi/viruses, enhanced sensitivity (68–90% *vs.* 20–30% positivity rates in sepsis), antibiotic-independent detection via direct nucleic acid amplification, and concurrent antimicrobial resistance profiling for precision therapy (He et al. 1998; Chiu and Miller 2019; Chen et al. 2022). Clinical validation by Langelier et al. (2018) demonstrated diagnostic potential of mNGS through integrated pathogen-microbiome-host analysis in acute respiratory failure patients, establishing its translational value for critical care respiratory infections.

In this study, all 30 patients with severe pneumonia complicated by bloodstream infection demonstrated pathogen positivity in BALF using mNGS. BALF mNGS identified 25 bacterial, 6 viral, 5 fungal, and 1 specific pathogen species. *K. pneumoniae*, *A. baumannii*, and *Stenotrophomonas maltophilia* emerged as predominant bacterial pathogens in our ICU cohort. Notably, *A. baumannii* accounts for 2–10% of Gram-negative infections in Western healthcare settings (Costa et al. 2006; Fournier et al. 2006), particularly affecting immunocompromised ICU patients with invasive device exposure. Similarly, *K. pneumoniae* is an opportunistic pathogen and has been identified as one of the most common causes of hospital- and community-acquired infections, including urinary tract infections, pneumonia, and intraabdominal infections (Zhang et al. 2016). It has developed resistance to various antibiotics, including tigecycline and carbapenem. *K. pneumoniae* has become one of the top 8 pathogens in hospitals worldwide due to its antibiotic resistance. *K. pneumoniae* is the most frequently detected pathogen in respiratory specimens and the second most common bacterium among all isolated strains (Zhou et al. 2022). *S. maltophilia* is an opportunistic pathogen that can lead to respiratory tract infections, urinary tract infections, trauma infections, surgical site infections, central nervous system infections, and sepsis (Patterson et al. 2020). *S. maltophilia* primarily affects immunocompromised patients or those on prolonged use of broad-spectrum antibiotics (Anđelković et al. 2019). Notably, clinical interpretation of mNGS results from non-sterile sites like BALF requires caution, as detected pathogens such as *A. baumannii* may represent colonization rather than true infection. Integrating imaging findings (e.g., chest X-ray or CT scan abnormalities indicative of active infection) and ventilation parameters (e.g., PaO_2/FiO_2 ratio, dynamic compliance, or plateau pressure trends) could enhance the specificity of pathogen attribution to clinical disease. Combined assessment with clinical indicators of respiratory decline or changing ventilation needs is essential to avoid overtreatment and antimicrobial resistance.

Current evidence suggests potential translocation of pulmonary microbiota into the bloodstream. However, this study observed significant discrepancies between pathogen profiles detected via BALF and plasma mNGS. Only 24/30 (80%) patients with severe pneumonia complicated with bloodstream infection had at least one plasma mNGS result that matched that of the BALF mNGS. Similar to the previous results (Chen et al. 2020), BALF samples demonstrated higher bacterial and fungal detection rates compared to blood samples,

likely reflecting the external exposure of airway versus physiological sterility of blood; conversely, blood mNGS exhibited superior viral detection sensitivity, potentially attributable to systemic viral shedding or reactivation from extra-pulmonary reservoirs.

In the study, the most commonly detected viral pathogens were Cytomegalovirus and Herpes simplex virus 1. These viral infections were particularly prevalent among immunocompromised patients. Herpes simplex virus 1 infections in these individuals can be severe and lead to extensive cutaneous or mucosal necrosis. Cytomegalovirus, a β-herpesvirus infecting most adults (Holder and Grant 2019), typically remains latent in healthy individuals but can cause life-threatening complications in immunosuppressed patients (Chiche et al. 2009), including interstitial pneumonia, hepatitis, and sepsis (Grahame-Clarke et al. 2003; Limaye and Boeckh, 2010).

In addition to common pathogens, *Rhizomucor pusillus* was identified in both BALF and blood mNGS of one patient. This fungus, a causative agent of mucormycosis, is classically associated with immunocompromised hosts, particularly those with diabetes, hematologic malignancies, or organ transplantation (Rawlinson et al. 2011; Gupta et al. 2023). However, the patient in our cohort lacked these traditional risk factors, including diabetes, chronic respiratory diseases, or immunosuppressive therapy. The detection of *R. pusillus* highlights the sensitivity of mNGS in identifying rare or atypical pathogens that may be overlooked by conventional methods. While mucormycosis is uncommon in non-immunocompromised individuals, its high mortality rate necessitates prompt recognition, even in the absence of typical comorbidities. Clinicians should consider integrating mNGS results with imaging and histopathological evaluations to confirm invasive fungal infections.

mNGS demonstrated superior diagnostic performance over conventional methods through direct microbial genomic analysis. BALF mNGS achieved 100% positivity versus 70% for traditional BALF testing ($p < 0.05$), while plasma mNGS showed 93.3% positivity compared to 10% in standard blood cultures ($p < 0.05$). This aligns with prior findings that 50% of septic shock patients yield negative blood cultures (Liu et al. 2021; Chumbita et al. 2022), exacerbated by antibiotic-induced microbial suppression reducing culture sensitivity (Cheng et al. 2019; Scheer et al. 2019). Sun et al. (2022) previously demonstrated antibiotic resistance of mNGS in lower respiratory tract infections, enhancing diagnostic accuracy. Our cohort's universal empirical antibiotic administration upon admission likely contributed to the diminished traditional detection rates observed. Besides, in complex cases, mNGS results should be interpreted alongside clinical status to guide anti-infective therapy decisions. Nevertheless, mNGS retains particular value in emergency settings including sepsis, immunocompromised hosts, and ICU epidemiology surveillance.

This study has several limitations. First, as a retrospective investigation with a limited sample size, it necessitates future large-scale prospective studies to validate the clinical utility of mNGS in diagnosing severe pneumonia complicated with bloodstream infections, thereby refining evidence-based diagnostic criteria. Second, using culture-based results as the reference standard for evaluating mNGS performance may have led to an underestimation of its sensitivity, given the potential for false-positive culture results. Third, prior antibiotic exposure likely reduced pathogen detection rates for both mNGS and conventional methods, although mNGS is comparatively less affected by antimicrobial therapy. Future prospective studies involving larger cohorts, integration of multi-omics data, and standardized protocols are warranted to corroborate these findings.

Conclusions

In summary, both blood and BALF-based mNGS demonstrate significant diagnostic utility for pathogen identification in severe pneumonia complicated by bloodstream infections. Findings from this study reveal that BALF mNGS exhibits superior sensitivity in detecting bacterial and fungal etiologies, whereas blood mNGS demonstrates enhanced capacity for identifying viral pathogens. This integrated approach may offer a novel diagnostic paradigm for critically ill patients with suspected severe pneumonia concurrent with bloodstream infection, future prospective studies with larger sample sizes are warranted to validate these findings. Furthermore, clinical implementation should incorporate mNGS results with patient-specific clinical context (e.g., respiratory deterioration or changes in ventilatory support requirements) to guide targeted anti-infective therapy and minimize inappropriate antimicrobial use in intensive care settings.

Abbreviations
mNGS – metagenomic next-generation sequencing
BALF – bronchoalveolar lavage fluid
DIC – disseminated intravascular coagulation
IDSA – Infectious Diseases Society of America
ATS – American Thoracic Society
ICU – Intensive Care Unit
AIDS – acquired immune deficiency syndrome

Availability of data and material
The data sets used and/or analyzed during the current study are available at National Genomics Data Center (http://ngdc.cncb.ac.cn), reference number PRJNA1101896.

Ethical statement
This research was approved by the ethics committee of First People's Hospital of Nanning. All patients signed the informed consent.

Authors' contributions
FML, FMX and QRZ designed the research and drafted the manuscript. XFL and QMY acquired data and analyzed data. YQL performed statistical analysis. CXH, QJH and LYX. JZ designed the research and revised manuscript for important intellectual content. All authors read and approved the final manuscript.

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Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Anđelković MV, **Janković SM**, **Kostić MJ**, **Živković Zarić RS**, **Opančina VD**, **Živić MŽ**. 2019. Antimicrobial treatment of *Stenotrophomonas maltophilia* invasive infections: systematic review. J Chemother. 31(6):297–306. https://doi.org/10.1080/1120009X.2019.1635209

Chen M, **Zhang M**, **Shi M**, **Hu X**. 2022. Diagnosis and analysis of clinical characteristics of *Chlamydia psittaci* pneumonia. Vector Borne Zoonotic Dis. 22(10):499–504. https://doi.org/10.1089/vbz.2022.0013

Chen X, **Ding S**, **Lei C**, **Qin J**, **Guo T**, **Yang D**, **Zhao F**, **Li J**, **Wang L**, **Wang Z**. 2020. Blood and bronchoalveolar lavage fluid metagenomic next-generation sequencing in pneumonia. Can J Infect Dis Med Microbiol. 2020:6839103. https://doi.org/10.1155/2020/6839103

Cheng MP, **Stenstrom R**, **Paquette K**, **Stabler SN**, **Akhter M**, **Davidson AC**, **Gavric M**, **Lawandi A**, **Jang W**, **Vinh DC**. 2019. Blood culture results before and after antimicrobial administration in patients with severe manifestations of sepsis: a diagnostic study. Ann Intern Med. 171(8):547–554. https://doi.org/10.7326/M19-1696

Chiche L, **Forel JM**, **Roch A**, **Guervilly C**, **Pauly V**, **Allardet-Servent J**, **Michelet P**, **Papazian L**, **Thomas P**, **Jaber S**. 2009. Active cytomegalovirus infection is common in mechanically ventilated medical intensive care unit patients. Crit Care Med. 37(6):1850–1857. https://doi.org/10.1097/CCM.0b013e31819ffea6

Chiu CY, **Miller SA**. 2019. Clinical metagenomics. Nat Rev Genet. 20(6):341–355. https://doi.org/10.1038/s41576-019-0113-7

Chumbita M, **Puerta-Alcalde P**, **Gudiol C**, **Garcia-Pouton N**, **Laporte-Amargós J**, **Ladino A**, **Cardozo C**, **Alonso-Fernández A**, **Royo-Cebrecos C**, **Carratalà J**. 2022. Impact of empirical antibiotic regimens on mortality in neutropenic patients with bloodstream infection presenting with septic shock. Antimicrob Agents Chemother. 66(2):e01744-21. https://doi.org/10.1128/AAC.01744-21

Cillóniz C, **Torres A**, **Niederman MS**. 2021. Management of pneumonia in critically ill patients. BMJ. 375:e065871. https://doi.org/10.1136/bmj-2021-065871

Costa GF, **Tognim MCB**, **Cardoso CL**, **Carrara-Marrone FE**, **Garcia LB**. 2006. Preliminary evaluation of adherence on abiotic and cellular surfaces of *Acinetobacter baumannii* strains isolated from catheter tips. Braz J Infect Dis. 10(5):346–351. https://doi.org/10.1590/S1413-86702006000500009

Evans L, **Rhodes A**, **Alhazzani W**, **Antonelli M**, **Coopersmith CM**, **French C**, **Machado FR**, **McIntyre L**, **Ostermann M**, **Prescott HC**. 2021. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Crit Care Med. 49(11):e1063–e1143. https://doi.org/10.1097/CCM.0000000000005337

Fournier PE, **Vallenet D**, **Barbe V**, **Audic S**, **Ogata H**, **Poirel L**, **Richet H**, **Robert C**, **Mangenot S**, **Abergel C**. 2006. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLoS Genet. 2(1):e7. https://doi.org/10.1371/journal.pgen.0020007

Grahame-Clarke C, **Chan NN**, **Andrew D**, **Ridgway GL**, **Betteridge DJ**, **Emery VC**. 2003. Human cytomegalovirus seropositivity is associated with impaired vascular function. Circulation. 108(6):678–683. https://doi.org/10.1161/01.CIR.0000084505.54603.C7

Gupta I, **Baranwal P**, **Singh G**, **Gupta V**. 2023. Mucormycosis, past and present: a comprehensive review. Future Microbiol. 18(3):217–234. https://doi.org/10.2217/fmb-2022-0211

He G, **Li H**, **Xu H**. 1998. The relationship between different serological types of *Pseudomonas aeruginosa* strain in lower respiratory tract and its clinical significance. Zhonghua Jie He He Hu Xi Za Zhi. 21(10):584–587.

Holder KA, **Grant MD**. 2019. Human cytomegalovirus IL-10 augments NK cell cytotoxicity. J Leukoc Biol. 106(2):447–454. https://doi.org/10.1002/JLB.2AB0418-158RR

Langelier C, **Kalantar KL**, **Moazed F**, **Wilson MR**, **Crawford ED**, **Deiss T**, **Belzer A**, **Bolourchi S**, **Caldera S**, **DeRisi JL**. 2018. Integrating host response and unbiased microbe detection for lower respiratory tract infection diagnosis in critically ill adults. Proc Natl Acad Sci U S A. 115(52):E12353–E12362. https://doi.org/10.1073/pnas.1809700115

Lanks CW, **Musani AI**, **Hsia DW**. 2019. Community-acquired pneumonia and hospital-acquired pneumonia. Med Clin North Am. 103(3):487–501. https://doi.org/10.1016/j.mcna.2018.12.008

Li Y, **Deng X**, **Hu F**, **Wang J**, **Liu Y**, **Huang H**, **Yu J**, **Wang L**, **Zhou Y**, **Zhang Y**. 2018. Metagenomic analysis identified co-infection with human rhinovirus C and bocavirus 1 in an adult suffering from severe pneumonia. J Infect. 76(3):311–313. https://doi.org/10.1016/j.jinf.2017.10.012

Limaye AP, **Boeckh M**. 2010. CMV in critically ill patients: pathogen or bystander? Rev Med Virol. 20(6):372–379. https://doi.org/10.1002/rmv.664

Liu L, **Maharjan S**, **Sun JL**, **Li YC**, **Cheng HJ**. 2021. Prevalence and clinical characteristics of septicemia in children with *Mycoplasma pneumoniae* pneumonia. J Int Med Res. 49(6):03000605211021733. https://doi.org/10.1177/03000605211021733

Patterson SB, **Mende K**, **Li P**, **Lu D**, **Carson ML**, **Murray CK**, **Akers KS**, **Be NA**, **Bishop-Lilly KA**, **Waterman PE**. 2020. *Stenotrophomonas maltophilia* infections: clinical characteristics in a military trauma population. Diagn Microbiol Infect Dis. 96(2):114953. https://doi.org/10.1016/j.diagmicrobio.2019.114953

Rawlinson NJ, **Fung B**, **Gross TG**, **Termuhlen AM**, **Skeens M**, **Garee A**, **Shenoy S**, **Mehta PA**. 2011. Disseminated *Rhizomucor pusillus* causing early multiorgan failure during hematopoietic stem cell transplantation for severe aplastic anemia. J Pediatr Hematol Oncol. 33(3):235–237. https://doi.org/10.1097/MPH.0b013e31820e3fd5

Scheer C, **Fuchs C**, **Gründling M**, **Vollmer M**, **Bast J**, **Bohnert JA**, **Zimmermann K**, **Hensel M**, **Kluge S**, **Keh D**. 2019. Impact of antibiotic administration on blood culture positivity at the beginning of sepsis: a prospective clinical cohort study. Clin Microbiol Infect. 25(3):326–331. https://doi.org/10.1016/j.cmi.2018.05.016

Shi Y, **Huang Y**, **Zhang TT**, **Cao B**, **Wang H**, **Zhuo C**, **Ye F**, **Su X**, **Fan H**, **Xu JF**. 2019. Chinese guidelines for the diagnosis and treatment of hospital-acquired pneumonia and ventilator-associated pneumonia in adults (2018 edition). J Thorac Dis. 11(6):2581–2616. https://doi.org/10.21037/jtd.2019.06.09

Sun H, **Wang F**, **Zhang M**, **Xu X**, **Li M**, **Gao W**, **Zhang H**, **Wang Y**, **Liu Y**, **Zhao Y**. 2022. Diagnostic value of bronchoalveolar lavage fluid metagenomic next-generation sequencing in severe pneumonia. Front Cell Infect Microbiol. 12:872813. https://doi.org/10.3389/fcimb.2022.872813

Walden AP, **Clarke GM**, **McKechnie S**, **Hutton P**, **Gordon AC**, **Rello J**, **Chiche JD**, **Stüber F**, **García-Salido A**, **Lipman J**. 2014. Patients with community-acquired pneumonia admitted to European intensive care units: an epidemiological survey of the GenOSept cohort. Crit Care. 18(2):R58. https://doi.org/10.1186/cc13812

Yang T, **Mei Q**, **Fang X**, **Zhu S**, **Wang Y**, **Li W**, **Li H**, **Chen X**, **Liu Y**, **Zhang Z**. 2022. Clinical value of metagenomics next-generation sequencing in bronchoalveolar lavage fluid for patients with severe hospital-acquired pneumonia: a nested case-control study. Infect Drug Resist. 15:1505–1514. https://doi.org/10.2147/IDR.S356662

Zhang R, **Lin D**, **Chan EWC**, **Gu D**, **Chen GX**, **Chen S**. 2016. Emergence of carbapenem-resistant serotype K1 hypervirulent *Klebsiella pneumoniae* strains in China. Antimicrob Agents Chemother. 60(1):709–711. https://doi.org/10.1128/AAC.02173-15

Zhou JJ, **Ding WC**, **Liu YC**, **Gao YL**, **Xu L**, **Geng RL**, **Li Y**, **Wang H**, **Zhang H**, **Liu X**. 2022. Diagnostic value of metagenomic next-generation sequencing for pulmonary infection in intensive care unit and non–intensive care unit patients. Front Cell Infect Microbiol. 12:929856. https://doi.org/10.3389/fcimb.2022.929856

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