Open Access Full Text Article

ORIGINAL RESEARCH

Clinical Application of Metagenomic Next-Generation Sequencing in Sepsis Patients with Early Antibiotic Treatment

Yongru Chen[1], Chongyue Chen[1], Wei Chen[2,3], Wei Gai[4], Yafeng Zheng[4], Yuxin Guo[4], Zhaoning Wang[1], Yongsong Chen[1], Zhiming Cai[1,5,6]

[1]The First Affiliated Hospital of Shantou University, Shantou, Guangdong, People's Republic of China; [2]Department of Urology, Shenzhen Institute of Translational Medicine, Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, International Cancer Center, Shenzhen University School of Medicine, Shenzhen, People's Republic of China; [3]Guangdong Key Laboratory of Systems Biology and Synthetic Biology for Urogenital Tumors, Shenzhen, People's Republic of China; [4]WillingMed Technology (Beijing) Co., Ltd, Beijing, People's Republic of China; [5]BGI Genomics, BGI Shenzhen, Shenzhen, People's Republic of China; [6]Carson International Cancer Center, Shenzhen University, Shenzhen, People's Republic of China

Correspondence: Zhiming Cai, Carson International Cancer Center of Shenzhen University, No. 1066, Xueyuan Road, Shenzhen, Guangdong Province, People's Republic of China, Tel +86 136 0253 0322, Email caizhiming2000@163.com; Yongsong Chen, The First Affiliated Hospital of Shantou University, No. 57, Changping Road, Shantou, Guangdong Province, People's Republic of China, Tel +86 0754-88905000, Email yongsongchen@126.com

Purpose: This study aimed to evaluate the clinical utility of metagenomic next-generation sequencing (mNGS) in sepsis patients who received early empirical antibiotic treatment.

Patients and Methods: A retrospective analysis was conducted on clinical data from sepsis patients diagnosed in the Emergency Intensive Care Unit (EICU) between April 2019 and May 2023. All patients underwent standard conventional microbiological testing. Patients were categorized into either the mNGS group or the control group based on whether they underwent mNGS tests. Baseline variables were matched using propensity scores.

Results: Out of 461 sepsis patients screened, 130 were included after propensity matching, with 65 patients in each group. Despite prior antibiotic treatment, 57 cases (87.69%) in the mNGS group had positive mNGS results, exceeding the culture detection rate (52.31%). Besides, a higher proportion of patients in the mNGS group experienced antibiotic adjustments compared to the control group (72.31% vs 53.85%). Mortality rates were also compared based on the duration of antibiotic exposure before mNGS sampling. Patients exposed to antibiotics for less than 24 hours had a lower mortality rate compared to those exposed for over 8 days (22.22% vs 42.86%). COX multivariate analysis identified mNGS testing, underlying diseases, lymphocyte percentage, infection site (respiratory and bloodstream) as independent risk factors for mortality in sepsis patients.

Conclusion: With increased antibiotic exposure time, the positive rate of culture testing significantly decreased (44.44% vs 59.52% vs 35.71%, P = 0.031), whereas the positive rate of mNGS remained stable (77.78% vs 88.10% vs 92.86%, P = 0.557). mNGS demonstrated less susceptibility to antibiotic exposure. Early mNGS detection positively impacted the prognosis of sepsis patients.

Keywords: metagenomic next-generation sequencing, sepsis, antibiotic management, clinical value, prognosis

Introduction

Sepsis is one of the leading causes of infection and mortality among critically ill patients worldwide.[1] Despite advances in antibiotic development and treatment approaches, the sepsis mortality rate remains persistently high.[2] Studies have shown that in sepsis patients with documented hypertension within the first 6 hours (h), each one-hour delay in administering effective antibiotics results in an average 7.6% decrease in survival.[3] Current guidelines strongly recommend that clinicians initiate broad-spectrum antibiotic therapy promptly, ideally within one hour of sepsis diagnosis, even in the absence of definitive pathogen identification.[4,5] This immediate treatment is essential in the Emergency Intensive Care Unit (EICU) and plays a crucial role in controlling disease progression. However, in real-world clinical practice,

Received: 19 August 2024
Accepted: 22 October 2024
Published: 26 October 2024

© 2024 Chen et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

inappropriate early empiric therapy may increase the risk of antibiotic resistance and toxicity, leading to higher mortality rates.[6] Therefore, timely and accurate pathogen identification is crucial for guiding early antimicrobial therapy, optimizing antibiotic stewardship, and clinical outcomes.[7]

Conventional culture methods remain the gold standard approach for pathogens identification in sepsis cases.[8] However, the positive rate of culture methods is often suboptimal, and further diminishes with prolonged empiric antibiotics use.[8–10] Although antigen/antibody assays and polymerase chain reaction (PCR) offer high sensitivity, they are often specific to certain pathogens and may miss rare or unexpected organisms. In recent years, metagenomic next-generation sequencing (mNGS) has rapidly advanced as a technology capable of detecting nearly all nucleic acid sequences in body fluid samples without bias,[11] providing greater precision and faster pathogen detection.[12–14] Moreover, mNGS exhibits superior sensitivity in diagnosing complex and severe infections and is less affected by prior antibiotic exposure.[15–17] However, accurate interpretation of mNGS results remains a significant challenge for clinicians,[11,18] and inappropriate antibiotic use in clinical practice can lead to adverse outcomes. Early targeted antibiotic administration, particularly in resource-limited settings such as the EICU, may improve the sepsis patient prognosis. This study aims to evaluate the performance of mNGS in early pathogen detection among sepsis patients with prior antibiotic exposure and to evaluate its impact on subsequent antibiotic adjustments.

Materials and Methods

Patient Enrolment and Study Design

A retrospective analysis was conducted on sepsis patients admitted to the EICU at the First Affiliated Hospital of the Medical College of Shantou University between April 2019 and May 2023. The inclusion criteria were: (1) patients met the sepsis 3.0 diagnostic criteria, jointly issued by the Society of Intensive Care Medicine (SCCM) and the European Society of intensive care Medicine (ESICM), and (2) the pathogen infection was unclear, no positive microbiological results were obtained from laboratory testing after admission, or infection symptoms did not improve following empiric treatment. Exclusion criteria were: (1) unqualified specimens or incomplete clinical data; (2) life expectancy of less than 24 h; (3) uncertain prognosis within 28 d.

Patients were divided into the mNGS group or control group based on whether mNGS testing was performed. In the mNGS group, specimens such as sputum, blood, bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), and pleural or peritoneal fluid were collected for culture and mNGS testing. Treatment decisions for these patients were guided by the mNGS results. In the control group, samples were solely utilized for routine tests, and treatment plans were based on conventional microbiological tests outcomes. The study was conducted in accordance with the Declaration of Helsinki.

Clinical Data Collection

Clinical data collected for these patients included demographic data, comorbidities, clinical indicators such as white blood cell count (WBC), percentage of neutrophils to total white blood cells (NE%), percentage of lymphocytes to total white blood cells (LY%), hemoglobin (Hb), platelet count (PLT), creatinine (Cr), alanine aminotransferase (ALT), lactate dehydrogenase (LDH)), procalcitonin (PCT) and C-reactive protein (CRP) levels. Additional data included the sequential organ failure assessment (SOFA) score, length of hospital stay (in days), antibiotic regimen, duration of antibiotic exposure prior to sampling (in days), ICU admission duration, and outcome. The primary outcome assessed was all-cause mortality within 28 d.

Nucleic Acid Extraction, Library Construction, and Sequencing

Nucleic acid was extracted with the PathoXtract® Basic Pathogen Nucleic Acid Kit (WYXM03211S, WillingMed Corp, Beijing, China) for DNA isolation and the PathoXtract® Virus DNA/RNA Isolation Kit (WYXM03009S, WillingMed Corp, Beijing, China) for RNA isolation following the manufacturer's protocol. Both DNA and RNA was eluted with 50 μL of nuclease-free water, combined, and the RNA underwent reverse transcription to complementary DNA using SuperScript® Double-Stranded cDNA Synthesis Kit (11917020, Invitrogen). For constructing

cfDNA (cell-free DNA) libraries from plasma, the KAPA DNA HyperPrep Kit (KK8504, KAPA, Kapa Biosystems, Wilmington, MA, United States) was used as per the manufacturer's guidelines. Genomic DNA libraries was prepared using the Illumina® DNA Prep, (M) Tagmentation (20018705, Illumina). The pooled libraries were sequenced on the NextSeq™ 550Dx system using a 75 bp, single-end sequencing kit (Illumina), ensuring a minimum of 20 million sequencing reads per sample.[19]

Bioinformatics Analysis

The raw FASTQ-format data underwent quality control and evaluation using Trimmomatic v0.40 to eliminate low-quality or undetected sequences, spliced sequences, high-coverage repeats, and short-read-length sequences.[20] The high-quality sequencing data were compared with the human reference genome GRCh37 (hg19) using Bowtie2 v2.4.3 to remove human host sequences.[21] The remaining sequences were aligned with the reference database using Kraken2 v2.1.0 to annotate pathogen genomes and identify pathogens in the samples.[22]

For pathogen identification, a RPTM (reads per ten million) value was used to identify positive pathogens, which defined as detected number of pathogen specific reads per ten million. A reads count ≥3 was employed for viral pathogen detection, while for bacteria and fungi, a threshold of RPMT ≥8 was applied in blood samples and sterile body fluids.[23,24] Non-sterile samples like BALF, sputum, and other samples required a higher RPTM threshold ≥20 for identifying positive bacteria and fungi. Notably, special pathogens (including Cryptococcus and Mycobacterium) with RPTM ≥ 1 was identified as positive.[24]

The average total readings produced by all samples, the readings produced by the host and the readings produced by the microorganisms were 37,097,326, 1,850,538 and 26,182,780, respectively.

Clinical Adjudication of mNGS Results

The mNGS results were reviewed by two infectious disease specialists. According to the standardized criteria of the Karius test study, the results of mNGS were divided into five categories: definite, probable, possible, unlikely, and false-negative results.[25] (1) Definite: mNGS results were found to be consistent with conventional microbiological tests (CMT) results performed within 7 days of mNGS detection; (2) Probably: microorganisms detected by mNGS may be the cause of infection; (3) Possible: Microorganisms detected by mNGS show that they may cause infection, but this was not a common cause based on the clinical expert's assessment based on the clinical record; (4) Unlikely: Based on other clinical outcomes, the microorganism detected by mNGS was not a possible cause of infection or the results are inconsistent with CMT; (5) False negative: The mNGS result was negative, but the case was assessed as infectious.[19,25,26]

Based on the final clinical evaluation, the categories of definite, probable, and possible was considered indicative of the disease cause, while unlikely pathogen were classified as a false-positives.

Criteria and Analysis of Antibiotic Changes

Following the microbiological tests results, antibiotic regimens were adjusted based on *The Sanford Guide to Antimicrobial Therapy*. For unsatisfactory clinical response: (A) If the current anti-infective regimen does not cover the pathogen, the appropriate antibiotics should be added. (B) If the current regimen covers the pathogen but drug resistance is suspected, therapy escalation therapy should be considered. For satisfactory clinical response: (A) If the pathogen is not covered by the current regimen, discontinuation of antibiotics is advised to prevent misuse. (B) If the pathogen is covered, therapy de-escalation should be implemented by transitioning from broad-spectrum to narrow-spectrum antibiotics.[27]

We evaluated the percentage of patients undergoing antibiotic de-escalation or escalation in both the mNGS and control groups. Antibiotic escalation involved adding at least one antibiotic or broadening the spectrum (from narrow-spectrum to broad-spectrum) in the treatment regimen. Antibiotic de-escalation entailed discontinuing at least one antibiotics or narrowing the spectrum (from broad-spectrum to narrow-spectrum) in the current treatment regimen. Meanwhile, "same level replacement" indicated no change in the number or spectrum of antibiotics.[28]

Statistical Analysis

The normality of distribution and homogeneity of variances were assessed using the *t*-test. Chi-square tests or Fisher's exact test were applied for categorical variables, while t-tests were used for continuous variables. To enhance the accuracy of NGS diagnostic efficacy, propensity score matching (PSM) was performed. Data analysis was conducted using SPSS 26.0 software (IBM, USA), with a *p*-value of less than 0.05 considered statistically significant. Independent risk factors for sepsis were identified using the Cox proportional hazards model, constructed with the "survival" package in R version v4.2.2.

Results

Baseline Characteristics of Participants

A total of 461 sepsis patients were enrolled in this study, with 78 in the mNGS group and 383 in the control group (Figure 1). Baseline characteristics for both groups were presented in Supplementary Table 1. Patients in the control group had significantly higher SOFA scores compared to those in the mNGS group (9 vs 5, $P < 0.001$). The most common infection site in both groups was the respiratory tract infection. Additionally, the control group showed a higher prevalence of genitourinary infections (22.72% vs 7.69%, $P = 0.002$), bloodstream infections (35.77% vs 20.51%, $P = 0.009$), and gastrointestinal infections (42.04% vs 11.54%, $P<0.001$), while the mNGS group had a higher prevalence of central nervous system (CNS) infections (8.97% vs 1.31%, $P = 0.001$). Hypertension was more prevalent in the mNGS group compared to the control group (48.72% vs 35.25%, $P=0.029$). Additionally, the control group exhibited significantly higher NE%, PCT and CRP levels than the mNGS group. Patients in the mNGS group had longer hospitalization and ICU stays compared to the control group. But they had a notably lower mortality rate (35.9% vs 51.44%, $P = 0.013$). There were no statistical differences between the groups for the remaining indicators.

A 1:1 PSM was performed for age, sex, SOFA score and underlying diseases to ensure comparable baseline characteristics between the two groups and reduce potential bias. After PSM, 65 patients were included in each group (Figure 1). While baseline characteristics were similar post-matching, some differences remained in specific variables (Table 1). A greater

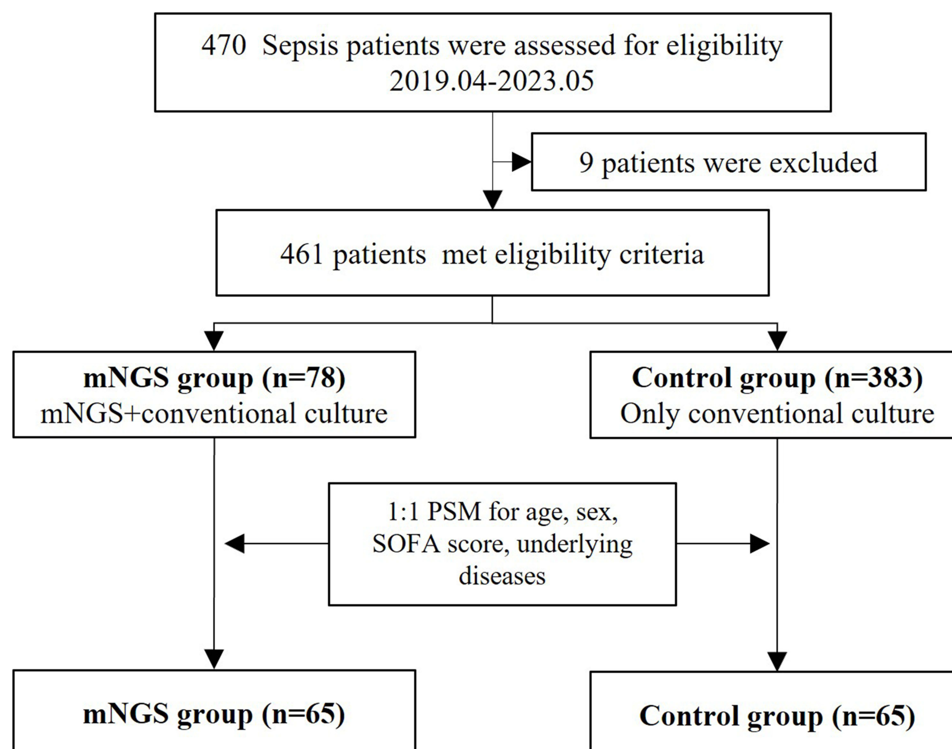

Figure 1 Flow diagram of patients enrolled in the study. Abbreviations: mNGS, metagenomic next-generation sequencing; PSM, propensity score matching, SOFA, sequential organ failure assessment.

Table 1 Demographics and Clinical Characteristics of Study Patients After PSM Balancing

Characteristic	mNGS Group (n=65)	Control Group (n=65)	*P*-value
Age, median (IQR)	64 (51–72)	65 (53–74)	0.659
Sex, n (%)			
Male	51 (78.46%)	44 (67.69%)	0.166
SOFA score, median (IQR)	5 (4–6)	6 (3–8)	0.076
Site of infection			
Respiratory	42 (64.62%)	38 (58.46%)	0.471
Genitourinary	4 (6.15%)	15 (23.08%)	0.006**
Skin and Soft Tissue	3 (4.62%)	5 (7.69%)	0.465
Bloodstream	15 (23.08%)	18 (27.69%)	0.546
CNS	6 (9.23%)	0 (0.00%)	0.012*
Gastrointestinal	8 (12.31%)	14 (21.54%)	0.161
Underlying diseases, n (%)			
Hypertension	29 (44.62%)	32 (49.23%)	0.598
Diabetes	24 (36.92%)	32 (48.57%)	0.122
CAD	15 (23.08%)	18 (27.69%)	0.546
CKD	7 (10.77%)	2 (3.08%)	0.084
Biochemical indicators			
WBC	12.46±9.05	11.72±6.65	0.594
NE%	59.61±40.04	82.74±11.45	<0.001***
LY%	8.84±16.14	11.06±10.26	0.350
Hb	105.58±28.47	110.57±26.05	0.300
PLT	167.52±92.80	207.83±111.52	0.027*
Cr	166.46±145.16	185.88±172.27	0.488
ALT	100.97±270.28	42.13±55.20	0.088
LDH	500.53±649.61	400.75±473.27	0.319
PCT	18.65±33.18	26.36±42.60	0.252
CRP	117.96±112.99	151.45±118.64	0.102
Outcomes, median (IQR)			
Hospital length of stay (days)	21 (9–34)	16 (10–28)	0.106
ICU length of stay (days)	10 (7–19)	6 (4–11)	0.002**
Mortality (%)	21 (32.31%)	23 (35.38%)	0.711
Duration of Antibiotic Exposure (Days, IQR)	3 (1–8)	——	——

Notes: The data were presented as n (%) or median (interquartile range, IQR). Differences with *P<0.05 were considered statistically significant. ** P<0.01, *** P<0.001.

Abbreviations: ALT, Alanine transaminase; CAD, coronary artery disease; CKD, Chronic Kidney Disease; CNS, Central Nervous System; Cr, Creatinine; CRP, C-reactive protein; Hb, hemoglobin; LDH, lactated hydrogenase; LY%, Percentage of lymphocytes and total white blood cell; mNGS, metagenomics next-generation sequencing; NE%, Percentage of neutrophils in blood; PCT, procalcitonin; PLT, blood platelet; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

proportion of patients in the control group developed sepsis due to genitourinary infection compared to the mNGS group (23.08% vs 6.15%, $P = 0.006$). Conversely, CNS infections were more common in the mNGS group than in the control group (9.23% vs 0.00%, $P = 0.012$). Patients in the mNGS group exhibited significantly lower NE% (59.61 ± 40.04 vs 82.74 ± 11.45, $P < 0.001$) and PLT levels (167.52 ± 92.80 vs 207.83 ± 111.52, $P = 0.027$) compared to the control group. Furthermore, ICU stays were also longer for mNGS patients (10 days vs 6 days, $P = 0.002$). No significant differences were observed between the two groups for the remaining baseline characteristics, underlying diseases, infection sites, biochemical indicators, or mortality.

Comparison of mNGS and Culture for Pathogen Detection

Of the 65 patients in the mNGS group, mNGS identified 57 positive cases (87.69%, 57/65), significantly surpassing the detection rate of culture (52.31%, 34/65, $P < 0.001$) (Figure 2A). Notably, mNGS had a higher positivity rate than culture across most sample types, except for BALF samples (Figure 2B). Patients were categorized into three groups based on

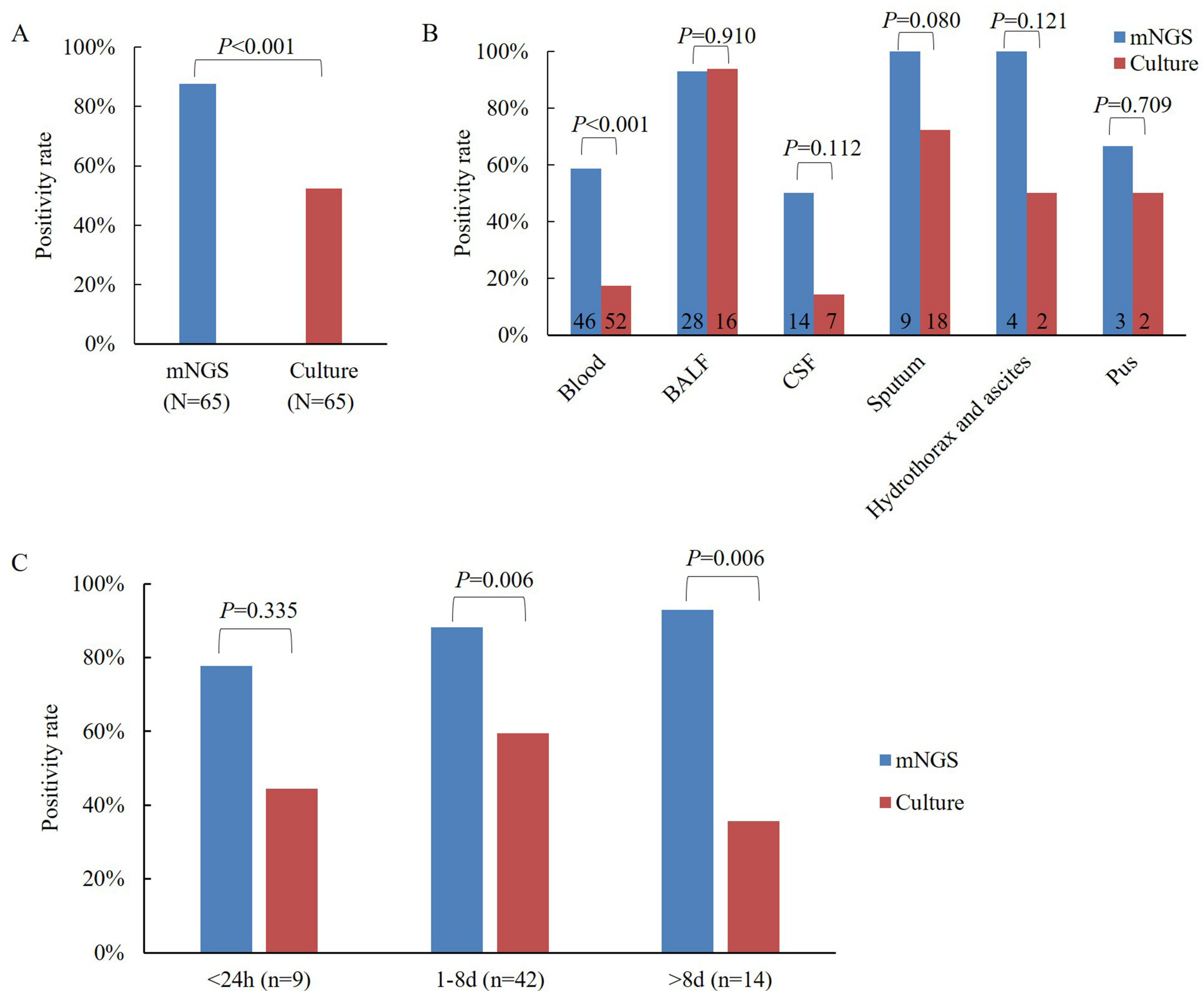

Figure 2 Comparison of pathogen positivity rates between mNGS and culture in the mNGS group. (**A**) Pathogens positivity rates identified by mNGS and culture in the mNGS group. (**B**) Pathogen positivity rates of mNGS and culture stratified by different sample types. (**C**) Positive rates of mNGS and culture stratified by different durations of antibiotic exposure. Abbreviations: BALF, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing.

the duration of antibiotic exposure prior to mNGS sampling: less than 24h, 1–8d, and more than 8d. Remarkably, no significant difference in mNGS positivity rate was observed among these groups (P = 0.557) (Figure 2C). Conversely, the culture positivity rate was significantly influenced by antibiotic exposure (P = 0.031), particularly in the group with more than 8d exposure, where the rate dropped to 35.71%. Across all time group, mNGS consistently showed a higher positivity rate than culture (Figure 2C).

mNGS detected a total of 79 pathogenic microorganisms, including 56 bacterial, 13 fungal, and 10 viral species (Supplementary Table 2). Culture identified 17 pathogenic microorganisms, including 15 bacteria and 2 fungi. Notably, 15 out of the 17 pathogens detected by culture were also identified by mNGS (Figure 3A). Among sepsis patients with prior antibiotic exposure, bacteria were the most frequently detected microorganisms, followed by fungi and viruses (Figure 3B). When combining results from both methods, the most detected bacteria were *Klebsiella pneumoniae* (16), *Escherichia coli* (10), *Enterococcus faecium* (8), and *Acinetobacter baumannii* (8), with the majority identified by mNGS (Figure 3C). Specifically, *Mycobacterium tuberculosis* complex (3) was exclusively detected by mNGS. *Candida albicans* and *Candida tropicalis* were the most prevalent fungi detected by both mNGS and culture methods. Furthermore, mNGS detected additional cases of *Candida glabrata* (3), *Aspergillus fumigatus* (3), and *Candida*

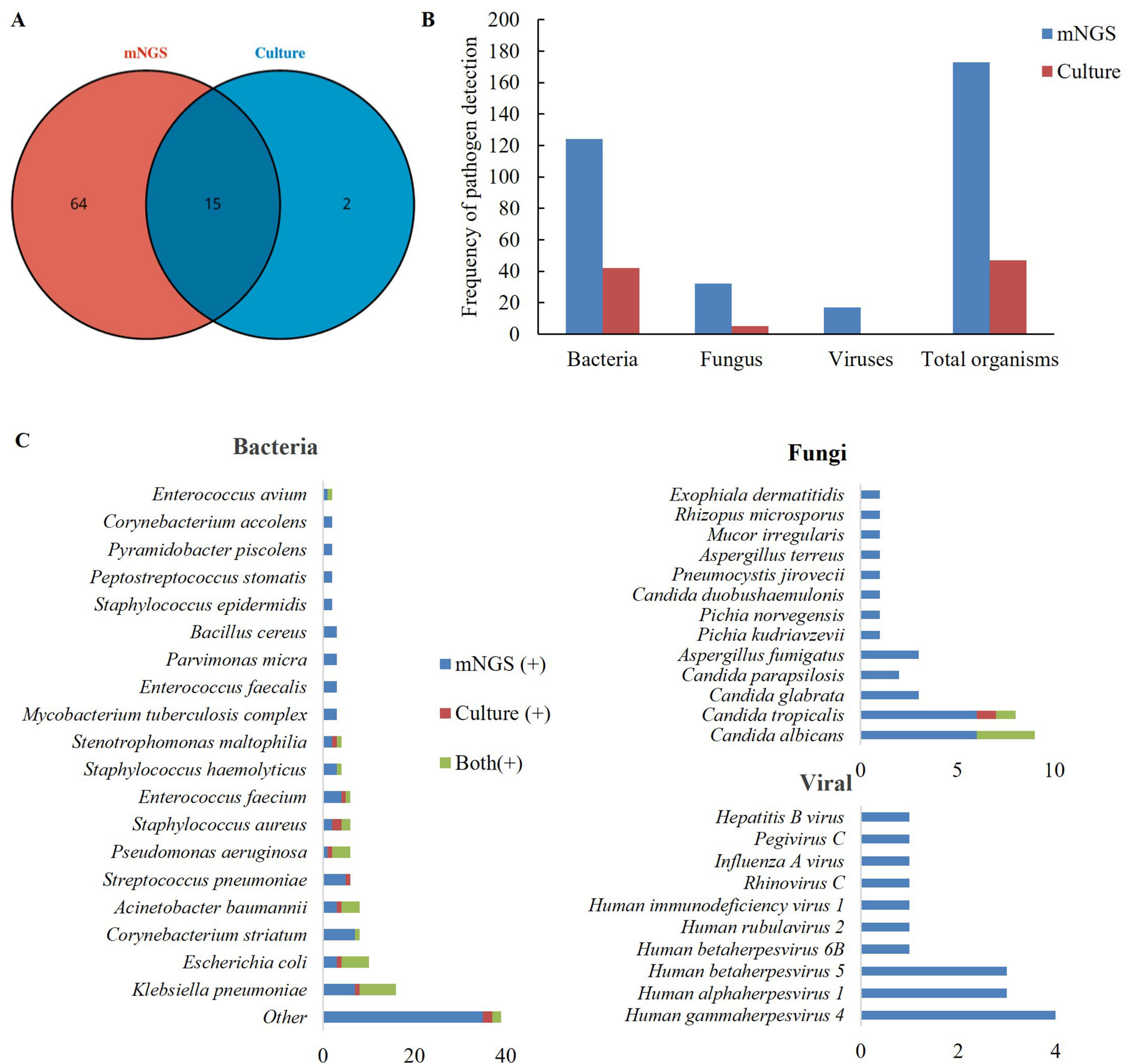

Figure 3 Pathogen distribution identified using mNGS and culture in the mNGS group. (**A**) Comparison of the quantity of pathogenic species identified by mNGS and culture detection. (**B**) Number of detection times for different types of pathogens identified by mNGS and culture detection. (**C**) Pathogens identified exclusively by mNGS, culture or both methods in the mNGS group.

parapsilosis (2). Since viral detection is not feasible through culture, only the viral results from mNGS were analyzed. The most detected viruses by mNGS were *Human gammaherpesvirus* 4 (4), *Human alphaherpesvirus* 1 (3), and *Human betaherpesvirus* 5 (3). Additionally, mNGS identified several RNA viruses in sepsis patients, including *Human immunodeficiency virus 1, Influenza A virus*, and *Rhinovirus C* (Figure 3C).

Comparison of Antibiotic Adjustments Between the mNGS and Control Group

The mNGS group had a significantly higher number of patients who adjusted their antibiotic regimens according to pathogen identification compared to the control group (P = 0.029) (Figure 4A and Supplementary Table 2). Among those who made adjustments, a larger proportion of patients in the control group increased antibiotic use compared to the mNGS group (28.57% vs 21.28%, P = 0.447) (Figure 4B and Supplementary Table 3). In contrast, the mNGS group had

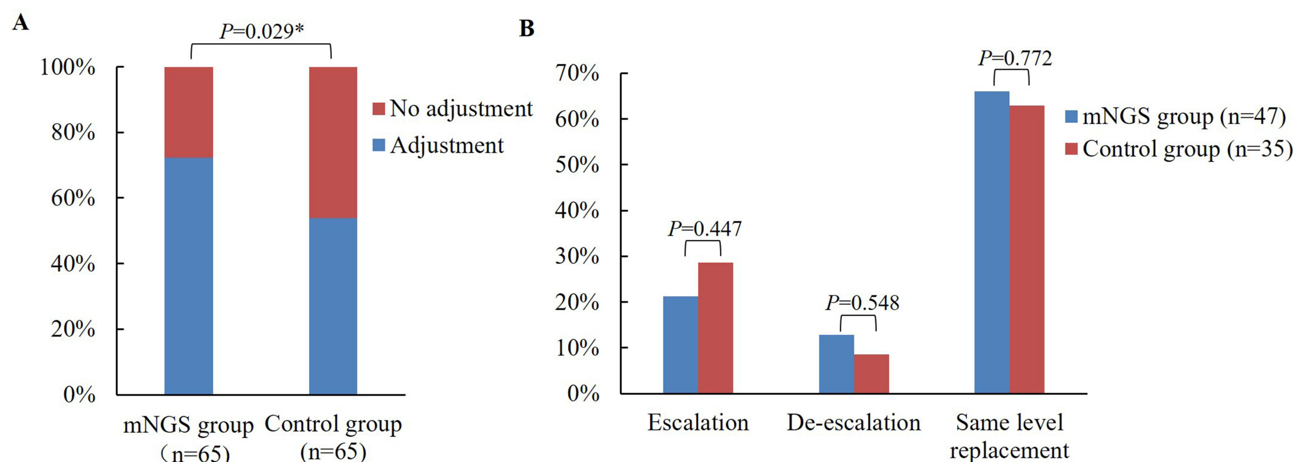

Figure 4 A comparison of antibiotic adjustments between the mNGS and control group. (**A**) Rates of antibiotic adjustments between the mNGS group and Control group based on the test results. (**B**) Forms of antibiotic adjustments in patients from the mNGS and control group who underwent antimicrobial adjustments. Differences with *P<0.05 were considered statistically significant.

a greater number of patients with reduced or de-escalated antibiotic regimens (12.77% vs 8.57%, *P* = 0.548). Furthermore, a higher percentage of patients in the mNGS group received targeted antibiotic substitution (65.96% vs 62.86%, *P* = 0.772). However, none of these differences reached statistical significance.

Impact of mNGS Sampling Time on the Prognosis of Sepsis Patient

The impact of mNGS sampling time on sepsis patient prognosis was evaluated by analyzing patients in the mNGS group with different durations of antibiotic exposure before sampling. Significant differences were observed in mortality rates, hospital stays, and ICU stays among the three exposure groups ($P < 0.05$) (Table 2). Our findings unveiled a positive correlation between longer antibiotic exposure and increased patient mortality, rising from 22.22% to 42.86%. Specifically, patients with less than 24 hours of antibiotic exposure had significantly shorter hospital stays and ICU stays compared to those exposed for more than 8 days.

Risk Factors Affecting Sepsis Patients Prognosis

To comprehensively evaluate the independent risk factors influencing the mortality of sepsis patients and the impact of mNGS testing on prognosis, we conducted a COX multivariate analysis. Variable included in the analysis were age, SOFA score, underlying diseases, absence of mNGS testing, culture positivity, WBC count, NE%, LY%, CRP, PCT levels, and infection sites (Table 3). The results identified the absence of mNGS testing (HR = 8.998, $P < 0.001$), presence of underlying diseases (HR = 2.916, $P = 0.042$), LY% (HR = 0.950, $P = 0.046$), respiratory tract infection (HR = 7.578, P=0.003), and bloodstream infection (HR = 7.246, P=0.003) as significant independent risk factors. No significant associations were found with the other parameters.

Table 2 Comparison of Outcomes with Different Durations of Antibiotic Exposure

Outcomes	<24h Group (n=9)	1–8d Group (n=42)	>8d Group (n=14)	P-value
Mortality (%)	22.22%	30.95%	42.86%	0.022
Hospital length of stay (days)	9 (4–12)	18 (9–28)	40 (24–83)	<0.001
ICU length of stay (days)	7 (4–8)	9 (7–15)	24 (22–40)	<0.001

Notes: The data were presented as median (interquartile range, IQR).

Table 3 Selection of Prognostic Risk Factors in Sepsis Patients Using a Multivariable Cox Proportional Hazards Model

Items	HR (95% CI)	*P*-value
Age	1.024 (0.999–1.054)	0.095
SOFA Score	0.983 (0.830–1.164)	0.844
Underlying diseases	2.916 (1.041–8.166)	0.042*
Not performing mNGS	8.998 (3.090–26.200)	<0.001***
Culture positive	1.394 (0.551–3.526)	0.483
WBC	0.983 (0.933–1.035)	0.506
NE%	0.989 (0.977–1.002)	0.102
LY%	0.950 (0.903–0.999)	0.046*
CRP	1.001 (0.997–1.004)	0.764
PCT	1.002 (0.992–1.012)	0.663
Respiratory tract infection	7.578 (2.018–28.447)	0.003**
CNS infection	6.695 (0.851–52.678)	0.071
Bloodstream infection	7.246 (1.970–26.651)	0.003**
Genitourinary infection	1.009 (0.314–3.249)	0.988
Gastrointestinal	1.052 (0.248–4.458)	0.945
Skin and Soft Tissue	1.709 (0.344–8.490)	0.512

Notes: Differences with *P<0.05 were considered statistically significant. ** P<0.01, *** P<0.001.
Abbreviations: HR, hazard ratio; CI, confidence interval.

Discussion

Our study aimed to compare the diagnostic efficacy of mNGS with culture in patients who had received prior empiric antimicrobial treatment. Furthermore, we assessed the impact of mNGS on antibiotic adjustments, prognosis, and sepsis risk factors in the EICU. The findings showed that mNGS demonstrated superior detection capabilities and was less affected by prior antimicrobial exposure. This early diagnostic advantage could enable more precise antibiotic management, consequently improving the prognosis of sepsis patients.

In the mNGS group, positivity rates for mNGS and culture were 87.69% and 52.31%, respectively. Interestingly, the mNGS positivity rate remained consistent regardless of the duration of prior antibiotic exposure time, whereas culture positivity significantly decreased in patients exposed to antibiotics for more than 8 days. This finding support that mNGS is less affected by antibiotic exposure compared to culture. Previous studies have similarly demonstrated the superior pathogen detection of mNGS, particularly in patients receiving antibiotic.[29,30] For example, a study on meningitis reported a considerable decrease in pathogen detection via CSF and blood cultures three days after antibiotic administration, while mNGS detection rates in CSF remained stable during the first five days of exposure, minimally affected by antibiotics treatment.[11] Similarly, Miao et al reported that mNGS maintained a higher positivity rate than culture in patients with prior antibiotic use.[15] These findings indicated the indispensable value of mNGS in diagnosing infections in patients with prior antibiotic exposure.

In our study, bacteria represented the largest proportion of pathogens, with *Klebsiella pneumoniae* and *Escherichia coli* being the most prevalent species, consistent with the epidemiology of sepsis in the ICU.[31,32] *Candida* and *Cryptococcus* were the predominant fungal pathogens, and mNGS exhibited superior sensitivity for detecting fungi compared to culture. This aligns with previous research highlighting the ability of mNGS in identifying fungi in patients receiving antifungal therapy.[33] The complexity of viral infections during sepsis is closely associated with the disease severity.[34] Additionally, mNGS has exhibited advantages in viral detection, including various RNA viruses and herpesviruses. While culture remains a cornerstone for diagnosing sepsis, mNGS provides rapid, unbiased pathogen detection, complementing clinical data.

Previous studies have often lacked clarity regarding the influence of mNGS on antibiotic management due to variability in the timing of empirical antibiotic treatment before mNGS testing. This inconsistency has affected decision-

making regarding antimicrobial therapy.[14] The use of mNGS has the potential to significantly influence clinical decisions by providing critical diagnostic insights. In our study, a higher percentage of patients in the mNGS group had their antibiotics adjusted based on mNGS findings compared to the control group (72.31% vs 53.85%). Among these patients, a higher proportion reduced or substituted antibiotics (78.73% vs 71.43%), with antibiotic reduction helping to prevent overtreatment and decrease healthcare burden, whereas substitution facilitated targeted therapy.[27] The data suggest that mNGS results can enhance the evaluation of empiric antimicrobial regimens, promoting targeted therapy, and improving patient prognosis by promptly adjusting antimicrobial treatment.[35]

Our study revealed a significantly lower mortality rate in the mNGS group compared to the control group before matching (35.9% vs 51.44%, $P = 0.013$). Post-matching, the mortality rate in the mNGS group remained lower, yet the difference was not statistically significant. Lu et al studied 158 patients with severe community-acquired pneumonia and found that those who received mNGS-guided therapy had a significantly lower mortality rate compared to patients treated with culture-based or empirical therapies.[36] Similarly, another study involving ICU patients with pneumonia found no significant difference in mortality between the mNGS and non-mNGS groups, but there was a trend toward reduced mortality in the mNGS group after PSM.[35] These findings, along with ours, indicate mNGS-based approaches may improve patient prognosis due to the faster and more accurate pathogen identification they provide.

Furthermore, our investigation demonstrated the advantages of early mNGS testing in reducing mortality. Patients who underwent mNGS within 24h of empirical antibiotic exposure experienced significantly lower mortality rates, shorter hospital stays, and reduced ICU stays compared to those exposed to antibiotics for over 8 days. Several studies have emphasized the importance of early, targeted treatment in improving patient outcomes and reducing mortality.[37] Therefore, we recommend conducting mNGS testing within 24h for individuals in severe conditions to achieve a more favorable prognosis.

We also conducted a risk factor analysis for sepsis patients in the EICU, providing valuable insights for patient management. Our analysis identified several independent risk factors for mortality, including mNGS testing, underlying diseases, LY% index, respiratory tract infections and bloodstream infections. Xi et al also found that the absence of mNGS testing was an independent risk factor for 28-day mortality in ICU patient with suspected infectious diseases undergoing mechanical ventilation.[37,38] Additionally, previous study has shown that decreased lymphocyte counts are associated with a three-fold increase risk of severe sepsis,[39] while underlying diseases have been identified as significant risk factors in bacterial sepsis patients.[40,41] An epidemiological study of sepsis patients in the Chinese ICU also identified specific infection sites (such as pneumonia and bloodstream infections) as risk factors for 90-day mortality in sepsis patients.[42] These studies strongly support the conclusions of our study.

However, our study has several limitations. First, due to the time-sensitive nature of sepsis diagnosis, not all patients underwent mNGS and culture testing from the same sample, although all tests were performed within 5 days. The lack of simultaneous sample collection may introduce variability between mNGS and culture results. Second, despite conducting propensity matching to equilibrate potential confounding factors, differences remained in the proportion of patients with genitourinary or CNS infections, as well as the prevalence of hypertension, which could affect the reliability of our results. Additionally, the allocation of patients to the mNGS and control groups were not fully randomized, as the decision was made by the patients themselves. Given the high cost of mNGS, patients with better financial status might have been more likely to choose mNGS, potentially introducing bias. The cost of mNGS also limited its broader application in sepsis patients, leading to a smaller sample size in the mNGS group. Therefore, our findings should be validated in larger prospective studies.

Conclusion

Our study indicates that mNGS can comprehensively identify pathogens in sepsis patients who have received early empirical antibiotic treatment. By guiding more precise antibiotic therapy, mNGS has the potential to significantly improve patient outcomes. We strongly recommend the timely use of mNGS testing in EICU sepsis patients who exhibit insufficient response to early empirical treatment.

Data Sharing Statement

The original contributions presented in the study are included in the article/Supplementary Materials Further inquiries can be directed to the corresponding authors.

Ethical Approval

The studies involving human participants were reviewed and approved by The Ethics Committee of Scientific Research and Clinical Trials of the First Affiliated Hospital of Shantou University (approval no. B-2023-254). Due to the retrospective nature of the study, the Ethics Committee waived the requirement for patient consents. The patients were anonymized, and their information was nonidentifiable.

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

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

Disclosure

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

References

1. Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing sepsis as a global health priority - A WHO resolution. *N Engl J Med*. 2017;377(5):414–417. doi:10.1056/NEJMp1707170
2. Ahn JY, Song JE, Ann HW, et al. Effects of early exercise rehabilitation on functional recovery in patients with severe sepsis. *Yonsei Med J*. 2018;59(7):843–851. doi:10.3349/ymj.2018.59.7.843
3. Ferrer R, Martin-Loeches I, Phillips G, et al. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program. *Crit Care Med*. 2014;42(8):1749–1755. doi:10.1097/ccm.0000000000000330
4. Evans L, Rhodes A, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med*. 2021;47(11):1181–1247. doi:10.1007/s00134-021-06506-y
5. Lo AH, Kee AC, Li A, Rubulotta F. Controversies in sepsis management-what is the way forward? *Ann Acad Med Singap*. 2020;49(9):661–668. doi:10.47102/annals-acadmedsg.202090
6. Li D, Gai W, Zhang J, Cheng W, Cui N, Wang H. Metagenomic next-generation sequencing for the microbiological diagnosis of abdominal sepsis patients. *Front Microbiol*. 2022;13:816631. doi:10.3389/fmicb.2022.816631
7. Mao JY, Li DK, Zhang D, Yang QW, Long Y, Cui N. Utility of paired plasma and drainage fluid mNGS in diagnosing acute intra-abdominal infections with sepsis. *BMC Infect Dis*. 2024;24(1):409. doi:10.1186/s12879-024-09320-1
8. Rajapaksha P, Elbourne A, Gangadoo S, Brown R, Cozzolino D, Chapman J. A review of methods for the detection of pathogenic microorganisms. *Analyst*. 2019;144(2):396–411. doi:10.1039/c8an01488d
9. Shangguan L, Xue L, Shang J, Wang H. The application value of metagenomic next-generation sequencing in community-acquired purulent meningitis after antibiotic intervention. *BMC Infect Dis*. 2023;23(1):683. doi:10.1186/s12879-023-08672-4
10. Pan TJ, Luo WW, Zhang SS, et al. The clinical application value of multi-site mNGS detection of patients with sepsis in intensive care units. *BMC Infect Dis*. 2024;24(1):920. doi:10.1186/s12879-024-09822-y
11. Wang L, Li S, Qin J, et al. Clinical diagnosis application of metagenomic next-generation sequencing of plasma in suspected sepsis. *Infect Drug Resist*. 2023;16:891–901. doi:10.2147/idr.S395700
12. Liang M, Fan Y, Zhang D, et al. Metagenomic next-generation sequencing for accurate diagnosis and management of lower respiratory tract infections. *Int J Infect Dis*. 2022;122:921–929. doi:10.1016/j.ijid.2022.07.060
13. Li X, Liang S, Zhang D, He M, Zhang H. The clinical application of metagenomic next-generation sequencing in sepsis of immunocompromised patients. *Front Cell Infect Microbiol*. 2023;13:1170687. doi:10.3389/fcimb.2023.1170687
14. Zuo YH, Wu YX, Hu WP, et al. The clinical impact of metagenomic next-generation sequencing (mNGS) test in hospitalized patients with suspected sepsis: a multicenter prospective study. *Diagnostics*. 2023;13(2):323. doi:10.3390/diagnostics13020323
15. Miao Q, Ma Y, Wang Q, et al. Microbiological diagnostic performance of metagenomic next-generation sequencing when applied to clinical practice. *Clin Infect Dis*. 2018;67(suppl_2):S231–s240. doi:10.1093/cid/ciy693
16. Didelot X, Bowden R, Wilson DJ, Peto TEA, Crook DW. Transforming clinical microbiology with bacterial genome sequencing. *Nat Rev Genet*. 2012;13(9):601–612. doi:10.1038/nrg3226
17. Huang J, Jiang E, Yang D, et al. Metagenomic next-generation sequencing versus traditional pathogen detection in the diagnosis of peripheral pulmonary infectious lesions. *Infect Drug Resist*. 2020;13:567–576. doi:10.2147/idr.S235182
18. Chen SX, Lin R, Shi JL, Lin W, Yu XF, Chen JY. Pathogenic profile characteristics and clinical risk factor analysis of patients who died from sepsis combined with pulmonary infection by metagenomic next-generation sequencing. *Infect Drug Resist*. 2023;16:7695–7705. doi:10.2147/idr.S415503
19. Xu X, Zheng Y, Zhang X, Zhang C, Gai W, Yang Z. Utility of metagenomic next-generation sequencing for diagnosis of infectious diseases in critically ill immunocompromised pediatric patients. *Infect Drug Resist*. 2024;17:3579–3591. doi:10.2147/idr.S472129

20. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114–2120. doi:10.1093/bioinformatics/btu170
21. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9(4):357–359. doi:10.1038/nmeth.1923
22. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol*. 2019;20(1):257. doi:10.1186/s13059-019-1891-0
23. Miller S, Naccache SN, Samayoa E, et al. Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid. *Genome Res*. 2019;29(5):831–842. doi:10.1101/gr.238170.118
24. Chen H, Zheng Y, Zhang X, et al. Clinical evaluation of cell-free and cellular metagenomic next-generation sequencing of infected body fluids. *J Adv Res*. 2023;2023. doi:10.1016/j.jare.2023.02.018
25. Blauwkamp TA, Thair S, Rosen MJ, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nat Microbiol*. 2019;4(4):663–674. doi:10.1038/s41564-018-0349-6
26. Xu C, Chen X, Zhu G, et al. Utility of plasma cell-free DNA next-generation sequencing for diagnosis of infectious diseases in patients with hematological disorders. *J Infect*. 2023;86(1):14–23. doi:10.1016/j.jinf.2022.11.020
27. Yan M, Zou X, Wang Y, et al. Metagenomic next-generation sequencing (mNGS) of bronchoalveolar lavage fluid on antimicrobial stewardship in patients with lower respiratory tract infections (LRTIs): a retrospective cohort study. *J Infect Dis*. 2023;28. doi:10.1093/infdis/jiad296
28. Moehring RW, Ashley ESD, Davis AE, et al. Development of an electronic definition for de-escalation of antibiotics in hospitalized patients. *Clin Infect Dis*. 2021;73(11):e4507–e4514. doi:10.1093/cid/ciaa932
29. Parize P, Muth E, Richaud C, et al. Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study. *Clin Microbiol Infect*. 2017;23(8):574.e1–574.e6. doi:10.1016/j.cmi.2017.02.006
30. Langelier C, Zinter MS, Kalantar K, et al. Metagenomic sequencing detects respiratory pathogens in hematopoietic cellular transplant patients. *Am J Respir Crit Care Med*. 2018;197(4):524–528. doi:10.1164/rccm.201706-1097LE
31. Vincent JL, Sakr Y, Singer M, et al. Prevalence and outcomes of infection among patients in intensive care units in 2017. *JAMA*. 2020;323(15):1478–1487. doi:10.1001/jama.2020.2717
32. Dugar S, Choudhary C, Duggal A. Sepsis and septic shock: guideline-based management. *Cleve Clin J Med*. 2020;87(1):53–64. doi:10.3949/ccjm.87a.18143
33. Camargo JF, Ahmed AA, Lindner MS, et al. Next-generation sequencing of microbial cell-free DNA for rapid noninvasive diagnosis of infectious diseases in immunocompromised hosts. *F1000Research*. 2019;8:1194. doi:10.12688/f1000research.19766.4
34. Walton AH, Muenzer JT, Rasche D, et al. Reactivation of multiple viruses in patients with sepsis. *PLoS One*. 2014;9(2):e98819. doi:10.1371/journal.pone.0098819
35. Liu Y, Zhang R, Yao B, et al. Metagenomics next-generation sequencing provides insights into the causative pathogens from critically ill patients with pneumonia and improves treatment strategies. *Front Cell Infect Microbiol*. 2022;12:1094518. doi:10.3389/fcimb.2022.1094518
36. Lu D, Abudouaini M, Kerimu M, et al. Clinical evaluation of metagenomic next-generation sequencing and identification of risk factors in patients with severe community-acquired pneumonia. *Infect Drug Resist*. 2023;16:5135–5147. doi:10.2147/idr.S421721
37. Xi Y, Zhou J, Lin Z, et al. Patients with infectious diseases undergoing mechanical ventilation in the intensive care unit have better prognosis after receiving metagenomic next-generation sequencing assay. *Int J Infect Dis*. 2022;122:959–969. doi:10.1016/j.ijid.2022.07.062
38. Li S, Zhou P, Yang L, et al. Clinical value of sampling time of metagenomic next-generation sequencing in patients with severe pneumonia. *Infect Drug Resist*. 2023;16:5263–5274. doi:10.2147/idr.S424185
39. Zhao Q, Meng M, Kumar R, et al. Lymphopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a systemic review and meta-analysis. *Int J Infect Dis*. 2020;96:131–135. doi:10.1016/j.ijid.2020.04.086
40. Fay K, Sapiano MRP, Gokhale R, et al. Assessment of health care exposures and outcomes in adult patients with sepsis and septic shock. *JAMA Network Open*. 2020;3(7):e206004. doi:10.1001/jamanetworkopen.2020.6004
41. Niederman MS, Baron RM, Bouadma L, et al. Initial antimicrobial management of sepsis. *Critical Care*. 2021;25(1):307. doi:10.1186/s13054-021-03736-w
42. Xie J, Wang H, Kang Y, et al. The epidemiology of sepsis in Chinese ICUs: a national cross-sectional survey. *Crit Care Med*. 2020;48(3):e209–e218. doi:10.1097/ccm.0000000000004155

Infection and Drug Resistance Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/infection-and-drug-resistance-journal