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Jingwei Tian
Sun Yat-sen Memorial Hospital
Jiwei Zhao
Sun Yat-sen Memorial Hospital
Xueru Chen
Sun Yat-sen Memorial Hospital
Zhifen Zeng
Sun Yat-sen Memorial Hospital
Haochen Mai
Sun Yat-sen Memorial Hospital
Tonghua Chen
Sun Yat-sen Memorial Hospital
Xiaojun Lei
Sun Yat-sen Memorial Hospital
Yuanfang Yu
Sun Yat-sen Memorial Hospital
Wenxia Wu
Sun Yat-sen Memorial Hospital
Liqiang Wen
Sun Yat-sen Memorial Hospital
Meng Zhang
zhangm7@mail.sysu.edu.cn

Sun Yat-sen Memorial Hospital

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Metagenomic Next-Generation Sequencing (mNGS) of Bronchoalveolar Lavage Fluid Versus Conventional Culture in Critically Ill Patients with Sepsis-Associated Pulmonary Infection: A Real-World Cohort Study

Jingwei Tian[1]*, Jiwei Zhao[1]*, Xueru Chen[2]*, Zhifen Zeng[1], Haochen Mai[1], Tonghua Chen[1], Xiaojun Lei[1], Yuanfang Yu[1], Wenxia Wu[1#], Liqiang Wen[1#], Meng Zhang[1,2#]

[1]Department of General Practice, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, P. R. China.

[2]Department of General Practice, Shenshan Medical Center, Sun Yat-sen Memorial Hospital, Shanwei, Guangdong, P. R. China.

*These authors contributed equally to this work

[#]Co-corresponding authors: Wenxia Wu (wuwenxia3@mail.sysu.edu.cn), Liqiang Wen (wenlq@mail.sysu.edu.cn), Meng Zhang (zhangm7@mail.sysu.edu.cn).

Abstract

Background: Sepsis-associated pulmonary infection in intubated patients remains a major diagnostic challenge in critical care, with conventional culture limited by low sensitivity, long

turnaround times, and inability to detect certain pathogens. Metagenomic next-generation sequencing (mNGS) offers unbiased, rapid pathogen detection. This study aimed to compare mNGS with conventional culture in terms of detection rates, turnaround time, and clinical impact on antimicrobial therapy.

Methods: A retrospective study was conducted on 92 intubated patients with ventilator-associated pneumonia (102 bronchoalveolar lavage fluid samples). All samples were tested simultaneously by mNGS and conventional bacterial/fungal culture. Detection rates, turnaround times, microbial species distribution, and the impact of mNGS results on antimicrobial modifications were assessed. Stratified analyses by patient outcome (improvement vs death) were performed to evaluate the association between mNGS-guided modifications and clinical prognosis.

Results: mNGS demonstrated significantly higher detection rates for bacteria (71.6% vs 52.0%, $p < 0.001$) and fungi (62.8% vs 42.2%, $p < 0.001$) compared with conventional culture. mNGS also detected viruses in 62.8% of samples. Turnaround time was markedly shorter for mNGS (mean 1.50 days) than for bacterial culture (2.94 days), fungal culture (4.67 days), and tuberculosis culture (23.5 days for the two samples tested, range 22–25 days). Based on mNGS findings in evaluable cases (n = 71), antibacterial therapy was modified in 71.8% of cases, while antifungal and antiviral modifications occurred in 31.0% and 9.9%, respectively. Stratified analysis showed that improved patients had consistently higher proportions of mNGS-guided modifications than deceased patients across bacterial (47.37% vs 34.44%) and fungal (52.63% vs 37.50%) infections, though differences were not statistically significant.

Conclusions: Compared with conventional culture, mNGS offers higher detection rates and faster turnaround, facilitating rapid diagnosis of complex infections in critically ill patients. In the

context of sepsis management in critical care, mNGS may serve as a rapid diagnostic adjunct to guide early targeted antimicrobial therapy. However, its clinical application for antibiotic guidance must balance these advantages against interpretive challenges and the need for clinical correlation. The potential prognostic benefit of mNGS-guided therapy, particularly in bacterial and fungal infections, warrants further investigation in larger prospective studies.

Keywords: metagenomic next-generation sequencing; sepsis; severe pneumonia; bronchoalveolar lavage fluid; antimicrobial stewardship

Background

Sepsis is a leading cause of mortality in intensive care units (ICUs), and the lungs are the most common source of infection in critically ill patients. Patients with severe pneumonia who are intubated in the ICU are at high risk for sepsis progression due to impaired airway defense mechanisms. Rapid and accurate pathogen identification is critical for guiding appropriate antibiotic therapy. Traditional culture has long been considered the diagnostic gold standard, but it has significant limitations, including long turnaround time (3–7 days), low sensitivity (positive rates of only 20%–40%)[1], and inability to culture certain pathogens (e.g., *Mycobacterium tuberculosis*, viruses). These limitations often result in delayed targeted treatment or unnecessary use of broad-spectrum antibiotics, which is particularly detrimental in sepsis, where each hour of delayed appropriate therapy is associated with increased mortality[2].

In recent years, metagenomic next-generation sequencing (mNGS) has emerged as a promising diagnostic tool that allows unbiased, high-throughput sequencing of all nucleic acids in a clinical sample[3,4]. This technology enables the simultaneous detection of bacteria, fungi, viruses, and parasites without prior knowledge of the pathogen, making it particularly useful in cases of sepsis, pneumonia, central nervous system infections, and other critical conditions where rapid diagnosis is essential[5]. At the same time, mNGS directly detects microbial nucleic acids in samples and can provide comprehensive pathogen profiles within 24–48 hours, making it particularly suitable for the rapid diagnosis of severe pneumonia in critically ill patients[6]. A large systematic review of 74 studies involving 10,566 patients found that mNGS identified pathogens in 78.9% of pneumonia cases compared to only 43.2% by conventional methods[7].

Despite its theoretical advantages, the integration of mNGS into routine clinical practice remains challenging, and high-quality evidence on its clinical utility in intubated patients is still lacking[8, 9]. In patients with severe pneumonia who require mechanical ventilation, any delay in pathogen identification may lead to inappropriate empirical therapy and increased mortality. Therefore, timely and accurate etiological diagnosis is important. However, key unanswered questions remain, including the concordance of mNGS with conventional cultures, its impact on clinical decision-making, and the degree of benefit from faster turnaround time in this specific population. Gao et al. demonstrated that BALF mNGS identified more pathogens than sputum culture, supporting the use of BALF as the preferred sample type[10]. Accordingly, this study aimed to perform a head-to-head comparison of mNGS and conventional culture using BALF samples in critically ill patients with sepsis-associated pulmonary infection, in terms of concordance, sensitivity, specificity, turnaround time, and therapeutic implications in a real-world clinical setting.

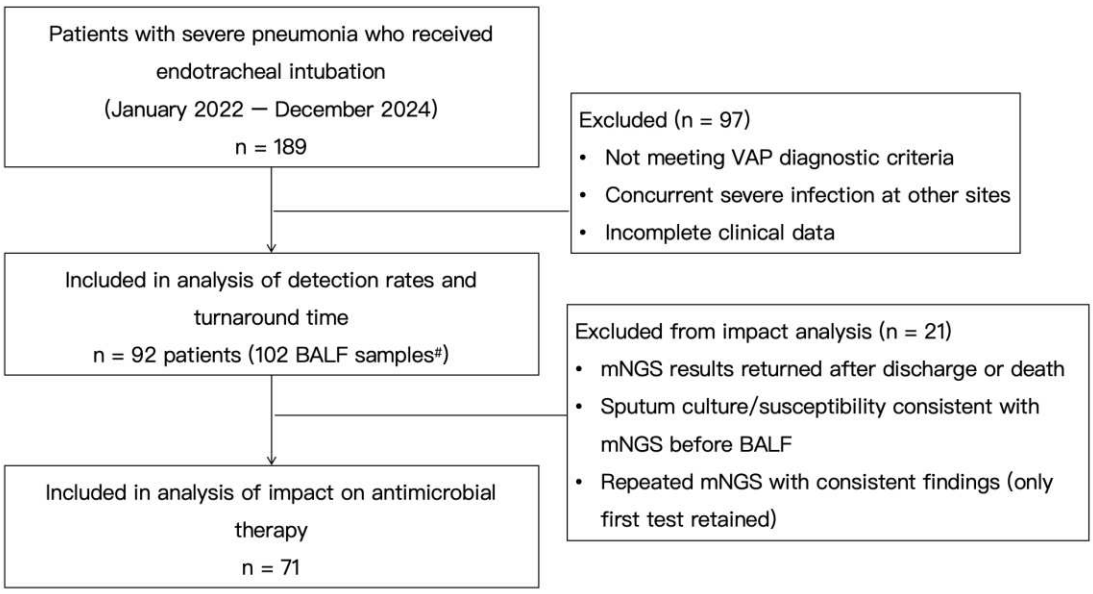
Methods

Study Population

Data from 189 critically ill patients with severe pneumonia, a leading source of sepsis in the ICU, who underwent endotracheal intubation in the ICU or emergency ICU of Sun Yat-sen Memorial Hospital, Sun Yat-sen University between January 2022 and December 2024 were retrospectively collected through the Hospital Information System (HIS) (Figure 1). The collected information included patients' sex, age, primary diagnosis, comorbidities, clinical outcomes, microbial species

identified from BALF samples tested by both mNGS and conventional culture, reporting times, and antibiotic usage. The study was approved by the Medical Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (approval No. SYSKY-2025-391-01).

Figure 1. Flow diagram of patient selection



#Some patients underwent repeated mNGS with interval >14 days and different predominant bacteria.

Inclusion criteria

a) Received mechanical ventilation (>48 hours) and met the diagnostic criteria for ventilator-associated pneumonia (VAP) recommended by the American Thoracic Society VAP expert conference[11], as a representative model of sepsis-associated pulmonary infection in critically ill patients.

b) Underwent bronchoscopy to obtain BALF;

c) BALF specimens were simultaneously tested by mNGS and conventional pathogen culture (for bacteria and fungi).

Exclusion criteria

a) Concurrent severe infections at other sites;

b) Incomplete clinical data;

c) For the analysis of clinical impact on antibiotic decisions, the following additional exclusions were applied: (1) patients who had been discharged or died by the time the mNGS report was returned; (2) patients who had sputum culture and susceptibility results consistent with the subsequent mNGS findings before BALF collection (as treatment would already have been guided); (3) patients with repeated mNGS testing within a short timeframe—only the first test was analyzed if repeated tests yielded consistent findings.

Assessment of mNGS Impact on Antimicrobial Therapy Decisions

The impact of mNGS results on antibiotic decision-making was categorized into three types based on physicians' responses after receiving the report: a) Positive impact: antimicrobial therapy was modified (including changing, adding, or discontinuing antibiotics, antifungals, or antivirals) based on mNGS findings. b) No impact: no change was made to the ongoing antimicrobial regimen after mNGS results were available. c) Negative impact: antimicrobial therapy was modified according to mNGS results, but subsequent conventional culture and susceptibility testing demonstrated resistance to the modified regimen, or for antivirals, the treatment was discontinued prematurely based on clinical judgment without completing the full course.

Testing Methods

This was a retrospective study, all tests were performed by the Bacterial/Fungal Culture

Laboratory and the Molecular Diagnostics Center of Sun Yat-sen Memorial Hospital. Reports for all specimens were issued by the same laboratory, ensuring methodological consistency.

Conventional Microbial Culture: Microbial cultures were conducted following standard clinical laboratory procedures. BALF samples were inoculated onto a range of culture media, including blood agar, chocolate agar, MacConkey agar, anaerobic blood agar, Sabouraud agar, potato dextrose agar, and/or chromogenic agar for *Candida*species, and incubated for a minimum of 48 hours[12].

mNGS Method: BALF samples were processed for host nucleic acid depletion, mechanical lysis, and total nucleic acid extraction. DNA and RNA libraries were prepared and sequenced on an Illumina platform. After bioinformatic removal of human reads, remaining sequences were aligned to a comprehensive microbial database (>25,000 species). A positive result was defined as detection of at least one unique read from a clinically relevant pathogen after excluding common contaminants[13, 14].

Statistical Analysis

Paired t-tests were used to compare turnaround time between mNGS and conventional culture. The chi-square test or Fisher's exact test was used to compare detection rates and impact proportions. A p value < 0.05 was considered statistically significant.

Results

Demographic Characteristics

A total of 92 patients with VAP were enrolled (Table 1). Among them, some patients underwent more than one mNGS test (with an interval >14 days and different predominant bacterial species), and a total of 102 samples were collected. The cohort consisted of 60 males (65.2%) and 32 females (34.8%), with a median age of 66.5 years (range, 24–95 years). All patients received mechanical ventilation (>48 hours) and met the ATS criteria for VAP. The median hospital length of stay was 23.0 days (range, 2–494 days). Regarding clinical outcomes, 41 patients (44.6%) were discharged against medical advice (most had severe disease and a poor prognosis), 32 (34.8%) died, and only 19 (20.7%) showed clinical improvement.

Table 1. Baseline demographic and clinical characteristics of the study population (N = 92)

Characteristic	Value
Age (years)	66.5 (24–95)
Gender, n (%)	
Male	60 (65.2%)
Female	32 (34.8%)
Length of hospital stay (days)	23.0 (2–494)
Disposition, n (%)	
Discharge against medical advice*	41 (44.6%)
Death	32 (34.8%)
Improved	19 (20.7%)

Note: Continuous variables (age and length of hospital stay) were not normally distributed and are presented as median (range). Categorical variables are presented as frequency (percentage).

*Most of these patients had severe disease and a poor prognosis.

Comparison of Detection Rates Between mNGS and Conventional Culture

As shown in Table 2, mNGS demonstrated significantly higher detection rates for bacteria (71.6%) and fungi (62.8%) compared with conventional culture (52.0% for bacteria, 42.2% for fungi; both $p < 0.001$). For*M. tuberculosis*, both methods detected 4 out of 102 samples (3.9%), with no significant difference ($p = 0.48$). mNGS also detected viral pathogens in 64 out of 102 samples (62.8%), whereas conventional culture does not routinely identify viruses.

Table 2. Comparison of detection rates between mNGS and conventional culture (N=102)

Pathogen	**mNGS (positive, %)**	**Culture (positive, %)**	***p* value**
Bacteria	73 (71.57%)	53 (51.96%)	<0.001
Fungi	64 (62.75%)	43 (42.16%)	<0.001
M. tuberculosis	4 (3.92%)	4 (3.92%)	0.480
Viruses	64 (62.75%)	Not applicable	-

Note: p values were calculated using the chi-square test.

Turnaround Time Comparison

The turnaround time (from sample collection to clinical reporting) was significantly shorter for mNGS (mean 1.50 ± 0.63 days) than for bacterial culture (2.94 ± 0.73 days; $p < 0.0001$) and fungal culture (4.67 ± 1.65 days; $p < 0.0001$) (Table 3). For the two samples tested for tuberculosis, the turnaround time was 23.5 days (range 22–25 days), whereas mNGS reported results within 1–2 days for those same samples.

Table 3. Turnaround time comparison between mNGS and conventional culture (N=102)

Methods	Turnaround time (days), mean ± SD	t value	*p* value
mNGS	1.50 ± 0.63	-	-
Bacterial culture	2.94 ± 0.73	-11.97	<0.0001
Fungal culture	4.67 ± 1.65	-12.07	<0.0001
M. tuberculosis culture	23.5 (22-25)[&]	-	-

Note: Turnaround time was defined as the interval from sample collection to reporting of results.

[&]For *M. tuberculosis* culture, only 2 samples were tested; data shown as range.

SD = standard deviation.

Microbial Species Distribution

The detected bacterial and fungal species are listed in Figure 2 and Figure 3. Among the bacteria isolated by mNGS, *Stenotrophomonas maltophilia* was the most frequently detected (15.7%), followed by *Pseudomonas aeruginosa* (10.5%), *Enterococcus faecium* (8.5%), *Staphylococcus aureus* (7.2%), *Escherichia coli* and *Klebsiella pneumoniae* (6.5% each). In contrast, conventional culture identified *S. maltophilia* as the most common (22.6%), followed by *P. aeruginosa* (9.4%), *E. faecium* (7.5%), *S. aureus* (5.7%), and *Klebsiella pneumoniae* (5.7%). For fungi, *Candida albicans* predominated in both methods (mNGS 35.5% vs culture 44.2%), followed by *Aspergillus fumigatus* (mNGS 14.5% vs culture 9.4%) and *Candida tropicalis* (mNGS 13.2% vs culture 7.5%). *Pneumocystis jirovecii* was detected only by mNGS (9.2%).

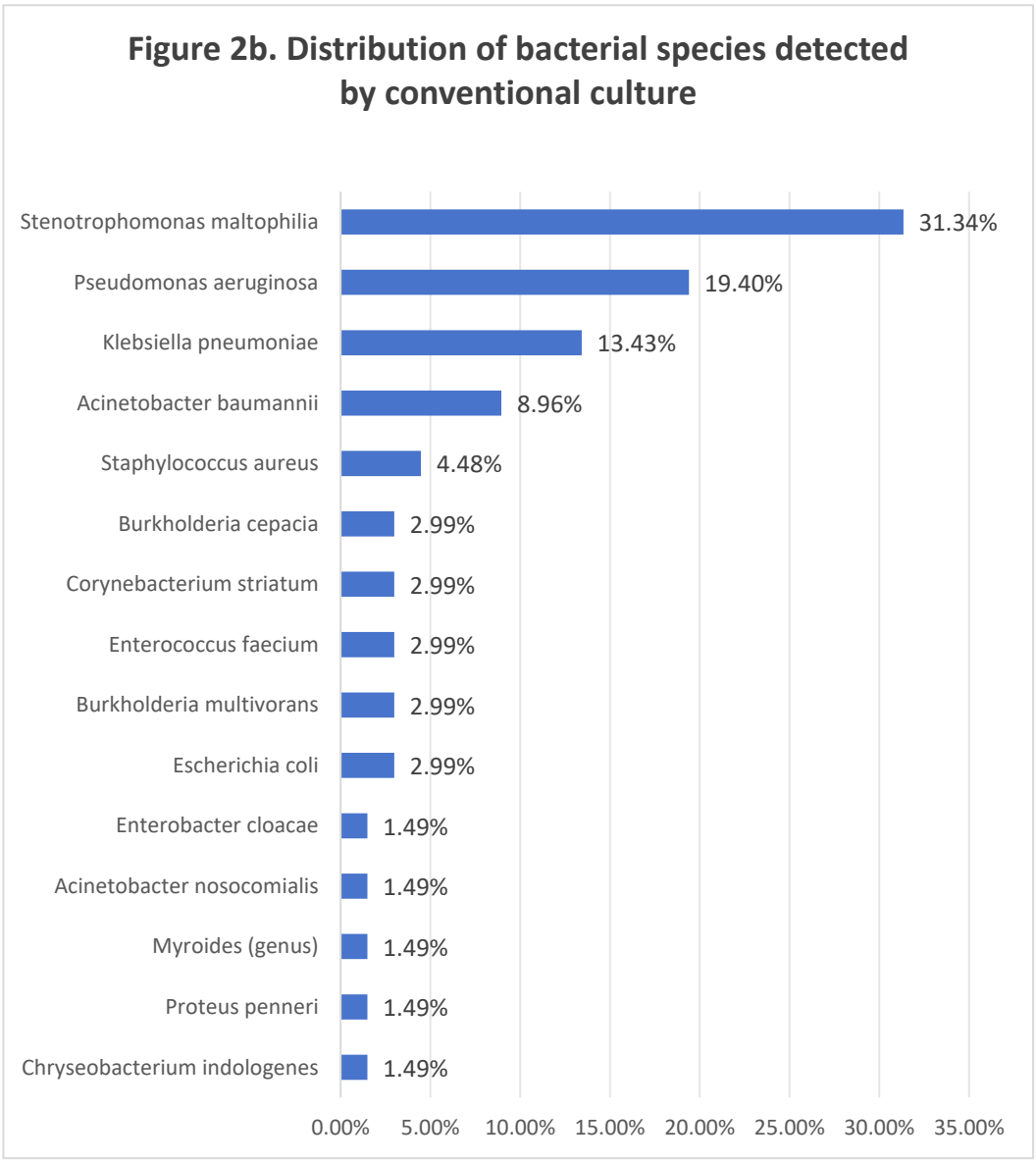
Figure 2. Distribution of bacterial species detected by mNGS (a) and conventional culture (b).

1

2

Figure 2a. Distribution of bacterial species detected by mNGS

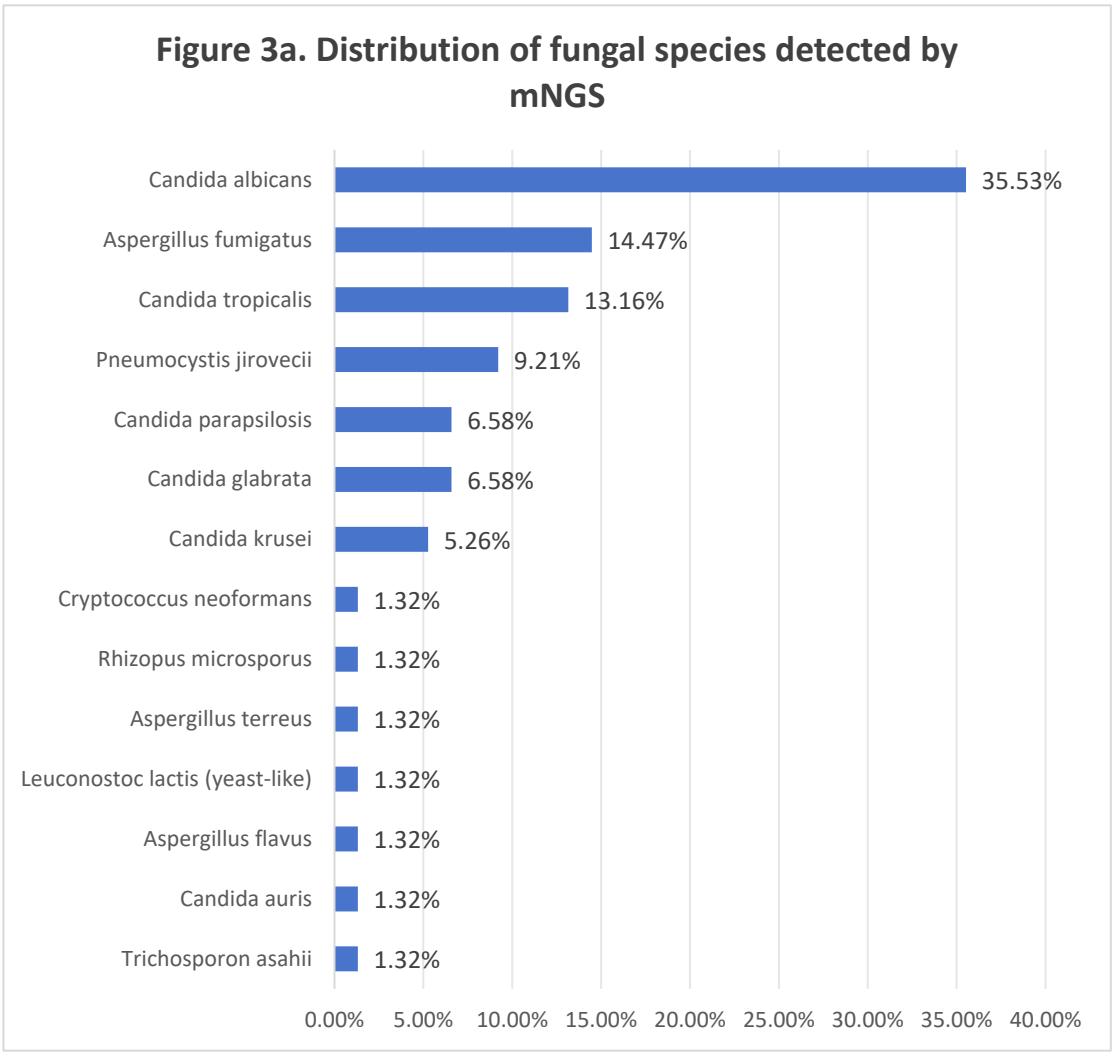


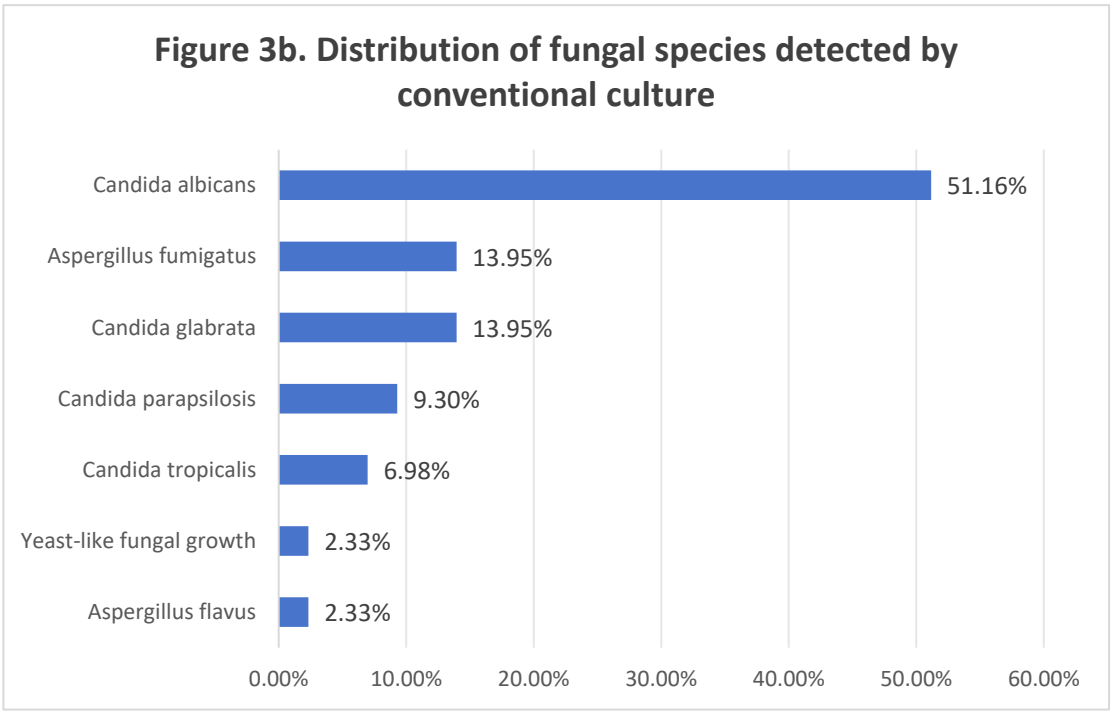

Figure 2b. Distribution of bacterial species detected by conventional culture
Stenotrophomonas maltophilia
Pseudomonas aeruginosa
Klebsiella pneumoniae
Acinetobacter baumannii
Staphylococcus aureus
Burkholderia cepacia
Corynebacterium striatum
Enterococcus faecium
Burkholderia multivorans
Escherichia coli
Enterobacter cloacae
Acinetobacter nosocomialis
Myroides (genus)
Proteus penneri
Chryseobacterium indologenes
31.34%
19.40%
13.43%
8.96%
4.48%
2.99%
2.99%
2.99%
2.99%
2.99%
1.49%
1.49%
1.49%
1.49%
1.49%
0.00%
5.00%
10.00%
15.00%
20.00%
25.00%
30.00%
35.00%

1

2

Figure 3. Distribution of fungal species detected by mNGS (a) and conventional culture (b).



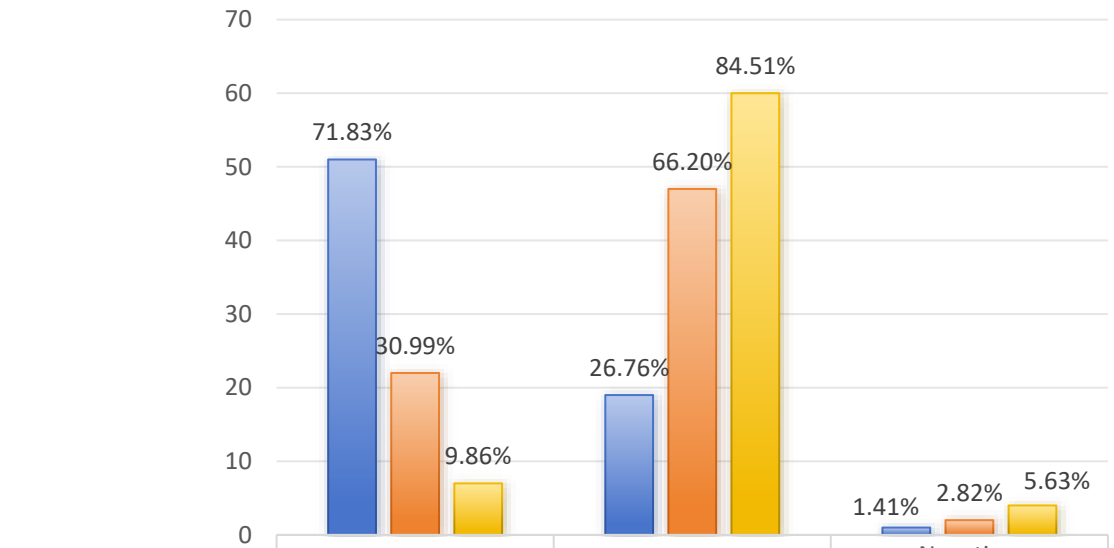

Figure 3b. Distribution of fungal species detected by conventional culture
Candida albicans
51.16%
Aspergillus fumigatus
13.95%
Candida glabrata
13.95%
Candida parapsilosis
9.30%
Candida tropicalis
6.98%
Yeast-like fungal growth
2.33%
Aspergillus flavus
2.33%
0.00%
10.00%
20.00%
30.00%
40.00%
50.00%
60.00%

Impact on Antimicrobial Therapy Decisions

A total of 71 cases were analyzed after exclusions. The impact of mNGS results on antimicrobial, antifungal, and antiviral decision-making is summarized in Figure 4. The mNGS findings led to modifications in antibacterial therapy in 51 cases (71.8%), with only one case (1.4%) showing subsequent resistance confirmed by culture and susceptibility testing. For antifungal therapy, modifications were made in 22 cases (31.0%), and negative impact (later resistance) was observed in 2 cases (2.8%). For antiviral therapy, modifications occurred in 7 cases (9.9%), while negative impact (premature discontinuation of antivirals based on clinical judgment before completing the full course) was noted in 4 cases (5.6%). In the majority of cases, mNGS results had no impact on antifungal (66.2%) or antiviral (84.5%) management.

Figure 4. Impact of mNGS results on antimicrobial therapy modifications.

Figure 4. Impact of mNGS results on antimicrobial therapy modifications (N=71)



	Positive impact	No impact	Negative impact
Antibacterial decision	51	19	1
Antifungal decision	22	47	2
Antiviral decision	7	60	4

Data are presented as the number of cases in which mNGS findings influenced clinical decision-making.

Positive impact: antibiotic therapy was changed, added, or discontinued based on mNGS results.

No impact: no modification was made after mNGS results were returned.

Negative impact: antibiotics were modified based on mNGS results, but subsequent culture and susceptibility testing demonstrated resistance to the modified regimen. For antivirals, negative impact included premature discontinuation based on clinical judgment without completing full course.

Stratified outcomes by mNGS impact type and patient disposition

To further characterize the relationship between mNGS-guided antimicrobial modifications and clinical outcomes, we stratified the 51 evaluable cases by patient disposition (improvement vs death) and compared the proportion of positive-impact cases—defined as antimicrobial therapy modified based on mNGS results—between the two groups (Table 4). In the improved group, the proportions of patients receiving mNGS-directed modifications were 47.37% (9/19) for bacteria, 52.63% (10/19) for fungi, and 15.79% (3/19) for viruses, compared with 34.44% (11/32), 37.50% (12/32), and 12.50% (4/32), respectively, in the death group. However, none of these differences reached statistical significance.

Table 4. Positive impact of mNGS on antimicrobial therapy: improved versus deceased patients

Pathogen category	Improved group (N = 19)	Death group (N = 32)	*p* value
Bacteria	9 (47.37%)	11 (34.44%)	0.358
Fungi	10 (52.63%)	12 (37.50%)	0.291
Viruses	3 (15.79%)	4 (12.5%)	0.714

Note: Data are presented as n (%), *p* values were calculated using the chi-square test or Fisher‘s exact test. Positive impact: antimicrobial therapy was modified based on mNGS results.

Discussion

In this real-world cohort of critically ill patients with sepsis-associated pulmonary infection, mNGS demonstrated substantially higher detection rates for bacterial and fungal pathogens compared with conventional culture, along with a significantly shorter turnaround time. These findings establish mNGS as a highly sensitive tool with clear diagnostic advantages over conventional methods.

Consistent with the literature, our study confirmed that mNGS offers superior detection rates for bacteria and fungi compared with conventional culture. A meta-analysis confirmed that mNGS substantially increases the pathogen-positive rate compared with conventional methods[8]. Several recent large-scale studies have further substantiated this finding. Zheng et al. analyzed 155 patients with lower respiratory tract infections and reported that mNGS achieved a positive detection rate of 81.29% compared to only 39.35% by conventional culture ($p < 0.05$), and mNGS directly led to treatment modifications in 37.42% of cases[15]. Jiang et al. similarly found, in a cohort of 369 patients with lower respiratory tract infections, that mNGS identified pathogens in 92.7% of cases compared to 58.8% by conventional microbiological tests, with significantly superior sensitivity (99.7% vs 57.1%) and negative predictive value (96.3% vs 6.3%)[16]. In immunocompromised patients with pneumonia, mNGS demonstrated diagnostic superiority across all infection types, detecting 98 cases versus 50 by conventional methods, and clinical outcomes improved in 45 patients following mNGS-guided therapeutic adjustments[17]. Another study reported that the overall positivity rate of mNGS was significantly greater than that of

conventional microbiological testing, with markedly higher sensitivity[18]. In a proof-of-concept study on hospital-acquired pneumonia/ventilator-associated pneumonia (HAP/VAP), Heitz et al. observed that all culture-positive BAL samples had concordant mNGS results, and additional bacterial species were revealed by mNGS in a substantial number of patients[19]. Similarly, Liu H. et al. demonstrated that mNGS detected a broader spectrum of pathogens compared with BALF culture, and a considerable proportion of cases were not covered by empirical antibiotics[6]. Our findings directly support these observations: mNGS not only identified 19.6% more bacterial-positive samples and 20.6% more fungal-positive samples than culture, but also detected viruses in 62.8% of samples, which culture cannot provide.

Our data demonstrated that mNGS offers a significantly shorter turnaround time compared with conventional cultures—approximately half that of bacterial culture and one-third that of fungal culture, with the most dramatic advantage over tuberculosis culture (23.5 days vs 1–2 days). This rapidity is particularly advantageous in the ICU setting, where delays in pathogen identification are associated with adverse outcomes. The clinical value of this accelerated timeline is supported by emerging evidence. A multicenter study of over 1,100 patients with severe pneumonia demonstrated that mNGS reports returned a median of 3 days earlier than culture results, and this earlier reporting was independently associated with a higher rate of antibiotic optimization within 48 hours (aOR = 2.31, 95% CI: 1.42–3.76) [6]. A randomized controlled trial focusing on the "time to result" endpoint showed that mNGS reduced the median time to pathogen identification from 53.4 hours (culture) to 22.8 hours ($P < 0.0001$), with the faster group achieving a significantly shorter time to clinical stability[20]. Collectively, these data suggest that the rapid turnaround time of mNGS can facilitate earlier therapeutic decisions, which is a critical

prerequisite for improving outcomes in critically ill patients. In sepsis, where delayed appropriate antimicrobial therapy is independently associated with mortality, this time advantage may be particularly relevant to early goal-directed therapy.

Our data also revealed notable differences in pathogen spectra between mNGS and conventional culture. Of note, *E. faecium* was detected much more frequently by mNGS than by culture (18.5% vs 7.5%), Does this raise the possibility that mNGS may detect colonization or contamination rather than true infection? *Enterococcus* species are often considered colonizers or contaminants in respiratory specimens, and current guidelines do not recommend anti-infective therapy based solely on the detection of *Enterococcus* in BALF in most patient populations[21]. However, in critically ill patients receiving broad-spectrum therapy, the presence of *Enterococcus* in BALF may reflect gut-lung translocation or prior antibiotic selection pressure[22]. A recent prospective study found that *E. faecium* is one of the leading ESKAPE pathogens causing ventilator-associated pneumonia, accounting for a substantial proportion of hospital-acquired infections[23]. Collectively, these findings suggest that *Enterococcus* should not be routinely dismissed as a mere colonizer or contaminant, and that mNGS may serve as a valuable complement to conventional culture results.

Regarding fungal distribution, *Candida albicans* predominated in both methods. While *Candida* is frequently detected in BALF, most cases represent airway colonization rather than invasive candidiasis. A study by Azoulay et al. demonstrated that presumptive antifungal therapy for BALF *Candida*-positive immunocompetent critically ill patients did not improve clinical outcomes, and current evidence does not support initiating antifungal therapy solely based on a positive BALF culture or mNGS result without strong clinical evidence of invasive disease[24]. In contrast, detection of *Aspergillus fumigatus* (14.5% by mNGS) carries greater clinical significance,

particularly in immunocompromised hosts. Zhong et al. conducted a multicenter study of 293 suspected IPA patients and reported that BALF mNGS achieved a sensitivity of 81.9% for the diagnosis of invasive pulmonary aspergillosis, significantly higher than BALF culture (27.0%, $p<0.001$) and BALF galactomannan (55.8%, $p<0.001$), while maintaining specificity comparable to conventional methods[25].

Another noteworthy finding is that Pneumocystis jirovecii was detected only by mNGS (9.2%). PJP is a severe opportunistic infection with specific therapeutic interventions—first-line treatment with trimethoprim-sulfamethoxazole—and diagnostic delay is associated with poor outcomes in critically ill patients[26], mNGS offers a significant diagnostic advantage for this otherwise unculturable pathogen. A dual-center study of 168 non-HIV patients reported that mNGS achieved a sensitivity of 100% and specificity of 96.4% for PJP diagnosis, markedly outperforming serum BDG testing (sensitivity 63.0%, specificity 90.4%)[27]. More importantly, this diagnostic advantage translates into actionable clinical benefit: in HIV-associated PJP patients, mNGS prompted treatment modification in 86.87% of cases, with 80% showing clinical improvement[28]; among immunocompromised non-HIV patients, mNGS-guided therapy adjustment was associated with improved 30-day survival[29]. In the dual-center study by Wang et al., 90.2% of mNGS-diagnosed PJP patients received specific anti-PJP therapy, and those who received targeted treatment had a significantly higher 30-day survival rate (91.8% vs 50.0%, $P = 0.003$)[26]. These data underscore that mNGS not only identifies a pathogen that conventional methods cannot culture, but also enables timely initiation of specific, life-saving therapy. mNGS should therefore be viewed as a rapid, sensitive screening tool most valuable when used in conjunction with culture and clinical assessment.

Our study showed that mNGS findings frequently led to modifications in antimicrobial therapy, supporting its clinical utility. Specifically, based on mNGS results in evaluable cases, antibacterial therapy was modified in 71.8%, antifungal therapy in 31.0%, and antiviral therapy in 9.9%. Liu H. et al. reported that a considerable proportion of mNGS-positive cases were not covered by empirical antibiotics, leading to antibiotic adjustments[6]. Zheng et al. found that treatment modifications guided by mNGS achieved beneficial clinical effects in 50.6% of immunocompromised CAP patients, with treatment confirmation in an additional 22.6%[30]. The meta-analysis by Lv et al. further demonstrated that mNGS-guided treatment adjustment was associated with improved outcomes[8]. In our study, only 1.4% of antibacterial modifications and 2.8% of antifungal modifications later showed resistance on conventional culture, suggesting that most mNGS-guided changes were appropriate.

The impact on antimicrobial stewardship is nuanced by the potential for false positives and unnecessary escalation. The low specificity of mNGS, as highlighted by Huang et al., raises the risk of unnecessary antibiotic escalation[18]. Zhang and Tian noted that mNGS positive results with low read counts pose significant interpretive challenges, and no quantitative threshold reliably distinguishes false positives from true positives[9]. In the proof-of-concept study by Heitz et al., mNGS revealed additional bacteria in culture-positive patients, but whether these required treatment or represented harmless colonization remained uncertain[19]. Furthermore, Li et al. reported that while mNGS was associated with lower rates of antibiotic escalation, it did not contribute to antibiotic de-escalation[31]. From a cost-effectiveness perspective, mNGS remains significantly more expensive than targeted sequencing methods, with one study reporting an mNGS cost of $840 compared to approximately half that for targeted approaches, although mNGS

identified the highest number of species[32, 33]. In our cohort, despite frequent modifications, we observed that mNGS results had no impact on antifungal therapy in 66.2% of cases and on antiviral therapy in 84.5% of cases, partly because empirical regimens already covered many detected fungi or because physicians judged viral findings as non-actionable. Thus, the use of antibiotics based on mNGS results requires comprehensive consideration of the clinical context, local epidemiology, and conventional confirmatory tests.

mNGS detected viral pathogens across a broad spectrum, providing etiological information entirely absent from conventional bacterial and fungal cultures. This capability is particularly valuable for rapid diagnosis of mixed viral-bacterial infections in severe pneumonia. Huang et al. identified multiple viral species using mNGS[18], and Liu H. et al. reported that mNGS detected viruses in a notable proportion of patients[6]. In our study, 62.8% of BALF samples were positive for at least one virus, with cytomegalovirus (CMV), Epstein-Barr virus (EBV), and herpes simplex virus (HSV) being the most common. However, the clinical significance of viral detection in intubated patients is often ambiguous, and the use of antiviral therapy requires comprehensive consideration of other factors. Without quantitative thresholds, it is difficult to differentiate true viral pneumonia from latent reactivation or incidental detection (e.g., CMV, HSV, EBV). In our study, 84.5% of viral detections did not lead to corresponding antiviral therapy, suggesting that the clinical significance of viruses detected by mNGS may be limited. Therefore, viral detection by mNGS should be considered a preliminary finding requiring clinical correlation and confirmatory testing such as quantitative PCR before initiating antiviral therapy.

Despite the absence of statistical significance, the consistently higher proportions of mNGS-guided modifications in the improved group—particularly for bacterial (47.37% vs

34.44%) and fungal (52.63% vs 37.50%) infections—suggest clinically meaningful differences of approximately 13% and 15%, respectively. The lack of significance is likely due to the small sample size (n = 19). This pattern—improved short-term responses without significant mortality reduction—has been observed in other limited-sample studies[20, 34, 35]. Conversely, when mNGS-guided treatment was effective, a multicenter study of immunocompromised SCAP patients reported significantly improved 28-day mortality[35], underscoring that benefit depends on actionable translation of results. These findings highlight the need for larger prospective studies—such as the ongoing NT-BALF trial—to definitively establish the survival benefit of mNGS-guided early targeted therapy in critically ill patients.

Conlusions

In summary, compared with conventional culture, mNGS offers higher detection rates and shorter turnaround times, enabling rapid diagnosis in intubated patients with severe pneumonia. Stratified analysis showed consistently higher proportions of mNGS-guided modifications in improved versus deceased patients for bacterial and fungal infections, suggesting a potential association with favorable outcomes. These findings support the potential role of mNGS as a rapid diagnostic tool within sepsis management bundles in critical care, although larger prospective studies are needed. However, clinicians must balance its high sensitivity against lower specificity and interpretive challenges. Larger prospective studies are warranted to define its optimal role and establish evidence-based algorithms for antimicrobial stewardship in ICU practice.

Declarations

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (approval No. SYSKY-2025-391-01).

Human Ethics and Consent to Participate declarations

Not applicable.

Consent for Publicaiton

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to hospital privacy regulations but are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

Meng Zhang, Liqiang Wen designed the study. Jingwei Tian, Jiwei Zhao, Xueru Chen, Zhifen Zeng, Haochen Mai, Tonghua Chen, Xiaojun Lei, Yuanfang Yu collected the clinical data. Jingwei Tian, Jiwei Zhao, Xueru Chen performed the statistical analysis and wrote the manuscript. Meng Zhang, Liqiang Wen and Wenxia Wu contributed to data interpretation and critically revised the manuscript. All authors read and approved the final manuscript.

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