Research article

# Clinical diagnostic value of metagenomic next-generation sequencing in patients with acute infection in emergency department

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ABSTRACT

*Objective:* To explore the value of metagenomic next-generation sequencing (mNGS) and culture in microbial diagnosis of patients with acute infection.
*Methods:* We retrospectively analyzed 206 specimens from 163 patients who were admitted to the emergency department of The First Affiliated Hospital of Sun Yat-sen University between July 2020, and July 2021. We evaluated the diagnostic efficacy of mNGS and in-hospital traditional culture.
*Results:* The total positive rate of mNGS was significantly higher than that culture methods (71.4 % vs 40.8 %, $p < 0.001$), while the sensitivity and accuracy of mNGS were found to be 92.9 % and 88.2 % respectively. However, culture exhibited superior specificity with a value of 92.6 % compared to 75.9 % for mNGS. The detection efficiency of mNGS and culture for fungi was comparable, but mNGS showed superior performance for bacterial detection. In the analysis of sepsis samples, mNGS outperformed traditional culture methods in diagnosing various types of samples, especially for sputum and bronchoalveolar lavage fluid. Among the identified infections, bacterial infections were the most common single infection (37.5 %). Additionally, bacterial-fungal infections represented the most prevalent form of mixed infection (77.3 %). *Candida albicans* and *Staphylococcus aureus* were identified as the predominant pathogens in the survival and death groups, respectively. No significant differences in microbial diversity were observed.
*Conclusion:* Compared to culture methods, mNGS demonstrates superior positive rates, sensitivity, and accuracy in the rapid detection of acute infections, particularly in critically ill patients such as those with sepsis. This capability establishes a foundation for the swift and precise identification of pathogens, allowing for the analysis of clinical indicators and patient prognosis based on the extensive data generated from mNGS.

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## 1. Introduction

Infectious disease is one of the most common diseases in the emergency department. In 2019, six of the top ten global health threats identified by the World Health Organization (WHO) were related to infectious diseases, including the global influenza pandemic, antimicrobial resistance, Ebola and other high-threat pathogens, vaccine hesitancy, dengue, and HIV [1]. It was estimated that the number of deaths attributed to infectious diseases that year was 13.7 million [1,2]. Severe infections can progress to sepsis, which further damage organ function and pose a life-threatening risk. It has the characteristics of rapid progression, difficult treatment and high mortality [3,4]. 40 % of patients are readmitted within 90 days of discharge, with 11.9 % of these readmissions attributed to infections [5]. Early empirical antibiotic treatment is often administered; however, 46 % of these treatments prove to be ineffective, contributing to 35 % of associated mortality. This situation increases the risk of drug resistance and toxicity, underscoring the urgent need for prompt infection detection [6]. Currently, culture is often regarded as the "gold standard", which remains the prevailing method for pathogen diagnosis in clinical settings. Other routine diagnostic methods, such as smear microscopy, serology and PCR, exhibit poor positive rates and may fail to provide timely diagnoses for uncommon or challenging-to-culture pathogens. Accurate and rapid pathogen identification can optimize antibiotic selection, thereby improving prognoses, increasing cure rates, and reducing hospital stays.

The application of mNGS in infectious diseases has drawn increasing interest and recognition as a novel technique that combines high-throughput sequencing with statistical analysis. By enabling the unbiased extraction and sequencing of nucleic acids from clinical samples and comparing biological data against the database, mNGS can simultaneously identify a wide range of pathogens, including bacteria, fungi, viruses and parasites. In comparison to conventional culture methods, mNGS offers significant advantages in terms of speed, sensitivity, and specificity [7,8]. The diverse infection sites and pathogens present in emergency patients complicate the determination of the underlying cause. Therefore, this research aims to evaluate the diagnostic efficacy of mNGS for identifying acute infection pathogens and to analyze the distribution of these pathogens.

## 2. Materials and methods

### 2.1. Study subjects

This study retrospectively collected data from patients admitted to the emergency department of the First Affiliated Hospital of Sun Yat-sen University, China, between July 1, 2020 and July 1, 2021. The inclusion criteria were as follows: (1) patients exhibiting typical clinical signs of infection, such as fever, cough, and body pain; (2) patient with available mNGS results and complete clinical data; (3) adherence to quality control protocols and sample testing criteria compatible with mNGS. The exclusion criteria included: (1) patients who did not undergo both concurrent culture and mNGS testing; and (2) patients with incomplete clinical documentation. Ultimately, a total of 206 samples were included in the analysis. The research process was illustrated in Fig. 1.
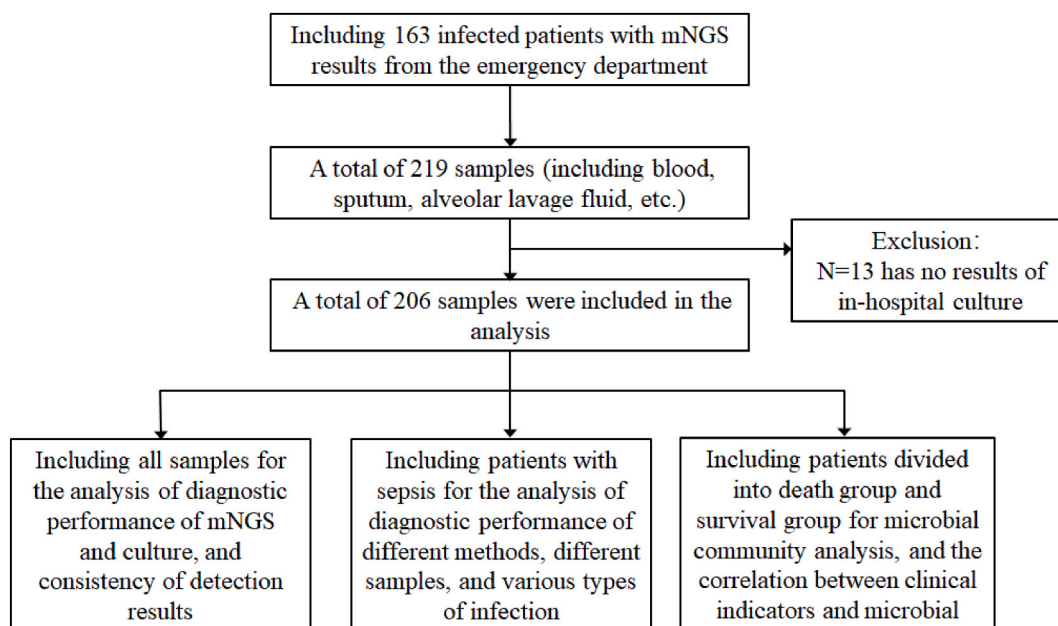


**Fig. 1.** The flow diagram of the study.

## 2.2. Collection of clinical data

The clinical data collection for the selected subjects included the following parameters: age, gender, medical history, length of hospital stay, clinical interventions (such as the use of vasoactive drugs, invasive ventilation, hemodialysis and antibiotics), and prognostic outcomes. The initial laboratory indicators assessed upon patient admission to the emergency department included: blood routine (WBC, NeuT #, Lyn #), inflammatory indicators (CRP, PCT), blood biochemistry (Glu, CO2, Lac, Amon), organ function indicators (TBIL, Cr), coagulation parameters (PT, Fbg) and LDH levels. Recording the results of routine detection in the hospital, including culture, sputum smear, fungal fluorescence, etc, along with details on the infection site, specimen type, specimen source, and pathogen results.

## 2.3. Specimen collection

Specimen of blood/sputum/bronchoalveolar lavage fluid (BALF)/urine/purulence were collected from patients according to standard procedures.

## 2.4. Nucleic acid extraction

Plasma was prepared from blood samples and circulating cell-free DNA (cfDNA) was isolated from plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. Sputum was liquefied by 0.1 % DTT (dithiothreitol) for 20 min at 56 °C before extraction. Other fluid samples were extracted using the TIANamp Magnetic DNA Kit (Tiangen, Beijing, China) according to the manufacturer's protocols.The quantity and quality of DNA was assessed using the Qubit (Thermo Fisher Scientific, Waltham, MA, USA) and NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA), respectively.

## 2.5. Library preparation and sequencing

DNA libraries were prepared using the Hieff NGS C130P2 OnePot II DNA Library Prep Kit for MGI (Yeasen Biotechnology) according to the manufacturer's protocols. And the library was constructed after purification.Agilent 2100 was used for quality control and DNA libraries were 50bp single-end sequenced on MGISEQ-200.

## 2.6. Bioinformatics analysis

Raw sequencing data was splited by removing low quality reads, adapter contamination, duplicated and shot (length<36 bp) reads. Human host sequence was subtracted by mapping to human reference genome (hs37d5) using bowtie2. Reads that could not be mapped to the human genome were retained and aligned with microorganism genome database for pathogens identification. Our microorganism genome database contained bacteria, fungi, virus and parasite genomic sequences (download from https://www.ncbi.nlm.nih.gov/).

## 2.7. Statistical treatment

The data were retrospectively processed and analyzed using SPSS 26.0 software. Measurement data conforming to a normal distribution were expressed as the mean ± standard deviation (x ± s), and the difference between groups was analyzed using the independent sample *T* test. The non-normally distributed measurement data were expressed as the median or interquartile range, and the non-parametric Mann-Whitney *U* test was used to examine the difference between groups. The enumeration data were statistically described by frequency and composition ratio (n, %), and $X^2$ or Fisher exact probability tests were used to compare the categories. At the pathogen level, the sensitivity, specificity and accuracy of different methods were calculated using the proportional standard formula, Statistical analysis was performed by R software (version 4.0.1). Alpha diversity was estimated by the Simpson index based on the taxonomic profile of each sample, beta diversity was assessed by the Bray-Curtis measure. $p < 0.05$ was regarded statistically significant for all two-sided statistical tests. The calculation formula of sensitivity, specificity and accuracy is as follows:

$$sensitivity = \frac{Ture\ Positive}{Ture\ Positive + False\ Negative} \quad Specificity = \frac{True\ Negative}{True\ Negative + Flase\ Positive}$$

$$Accuracy = \frac{Ture\ Positive + True\ Negative}{Total\ samples}$$

## 3. Results

### 3.1. Patient baseline characteristics

The median age of the 163 patients with emergency infection included in this study was 61 (IQR: 42–70). Of these patients, 105 patients were males (64.4 %) and 108 (66.3 %) patients presented with fever as their primary symptom. The median SOFA score was

four points (Table 1). In the basic medical history, hypertension (54, 33.1 %), tumor history (52, 31.9 %), cardiovascular disease (49, 30.1 %), immunosuppressive diseases (42, 25.7 %), and diabetes (39, 23.7 %) were significantly more prevalent with emergency infection. Among the infection sites, the most common infection site was lung (125, 77.2 %), followed by urinary tract (29, 17.9 %), digestive tract (24, 14.8 %), blood (18, 11.1 %) and skin and soft tissue (8, 4.9 %). Among treatment and prognostic indicators, the median overall hospital stay was 12 days. During their hospitalizations, most patients were treated with vasoactive drugs (38.0 %), invasive ventilator ventilation (33.1 %), and hemodialysis (19.6 %) during hospitalization. Finally, 16 (9.8 %) patients died.

### 3.2. Clinical Diagnostic Effect of mNGS and culture

A total of 206 specimens were tested for mNGS and in-hospital culture, including 113 blood samples (54.9 %), 39 sputum specimens (18.9 %), 33 samples of bronchoalveolar lavage fluid (16.0 %), 20 urine specimens (9.7 %), and 1 drainage fluid sample (0.5 %). The total positive rate of mNGS was 71.4 %, which was significantly greater than that of culture (40.8 %, $p < 0.001$). Except for the drainage fluid, the positive rate of mNGS in all types of specimens was higher than that of traditional culture. The positive frequencies of mNGS were significantly higher compared with conventional culture in various types of specimens: blood (50.4 % vs 19.5 %, $p < 0.001$), sputum (100 % vs 79.5 %, $p = 0.003$), bronchoalveolar lavage fluid (93.9 % vs 69.7 %, $p = 0.011$), and urine specimens (95.0 % vs 39.8 %, $p < 0.001$). Due to the singular occurrence of the drainage fluid sample, statistical evaluation was not performed for that data point. (Fig. 2A).

Subsequently, an analysis was conducted on the 195 samples following the exclusion of 11 with unclear clinical diagnoses. As illustrated in Fig. 2B, when comparing against the final clinical diagnosis as the reference standard, mNGS demonstrated superior sensitivity and accuracy, yielding values of 92.9 % and 88.2 %, respectively. In contrast, traditional culture exhibited sensitivity and accuracy of only 56.7 % and 66.6 %, respectively. However, it should be noted that mNGS showed comparatively low performance in

**Table 1**
Demographic characteristics and outcomes of included patients.

| Characteristics | Value |
|---|---|
| Age, mean ± SD (y) | 61 ± 19.1 |
| Age group (y), n(%) | |
| <18 | 4(2.4 %) |
| 18-44 | 39(23.9 %) |
| 45-64 | 57(35.0 %) |
| ≥65 | 63(38.7 %) |
| Gender, male, n (%) | 105(64.4 %) |
| Fever, n (%) | 108(66.3 %) |
| SOFA score | 4(1–8) |
| Basic medical history | |
| Immunocompromised host, n (%) | 42(25.7 %) |
| Tumor, n (%) | 52(31.9 %) |
| Cardiovascular disease, n (%) | 49(30.1 %) |
| Respiratory diseases, n (%) | 22(13.4 %) |
| Hypertension, n (%) | 54(33.1 %) |
| Diabetes, n (%) | 39(23.9 %) |
| Infection site | |
| Lung, n (%) | 125(77.2 %) |
| Blood, n (%) | 18(11.1 %) |
| Digestive tract, n (%) | 24(14.8 %) |
| Urinary tract, n (%) | 29(17.9 %) |
| Skin soft tissue, n (%) | 8(4.9 %) |
| Laboratory index | |
| WBC, median [range], $\times 10^9$/L | 10.05(6.35–15.49) |
| NEUT#, median [range], $\times 10^9$/L | 8.39(5.21–13.28) |
| LY#, median [range], $\times 10^9$/L | 0.88(0.48–1.33) |
| CRP, median [range], mg/L | 116.75(20.35–195.9) |
| PCT, median [range], ng/mL | 1.05(0.22–6.64) |
| PT, median [range], s | 14.7(13.3–16.5) |
| FIB, median [range], g/L | 4.41(2.95–6.36) |
| TBIL, median [range], umol/L | 15.4(10.4–25.3) |
| CREA, median [range], umol/L | 82(60–217) |
| LDH(U/L) | 284(198–495) |
| Treatment indicators | |
| Vasoactive drugs, n (%) | 62(38.0 %) |
| Invasive ventilator assisted ventilation, n (%) | 54(33.1 %) |
| Hemodialysis, n (%) | 32(19.6 %) |
| Total length of stay, median [range], days | 12(7–21) |
| Mortality, n (%) | 16(9.8 %) |

WBC, White Blood Count; NEUT#, Neutrophil count; LY#, Lymphocyte count; CRP, C-reactive protein; PCT, Procalcitonin; PT; Prothrombin Time; FIB, Fibrinogen; TBIL, Total Bilirubin; CREA, Creatinine; LDH, Lactate Dehydrogenase.
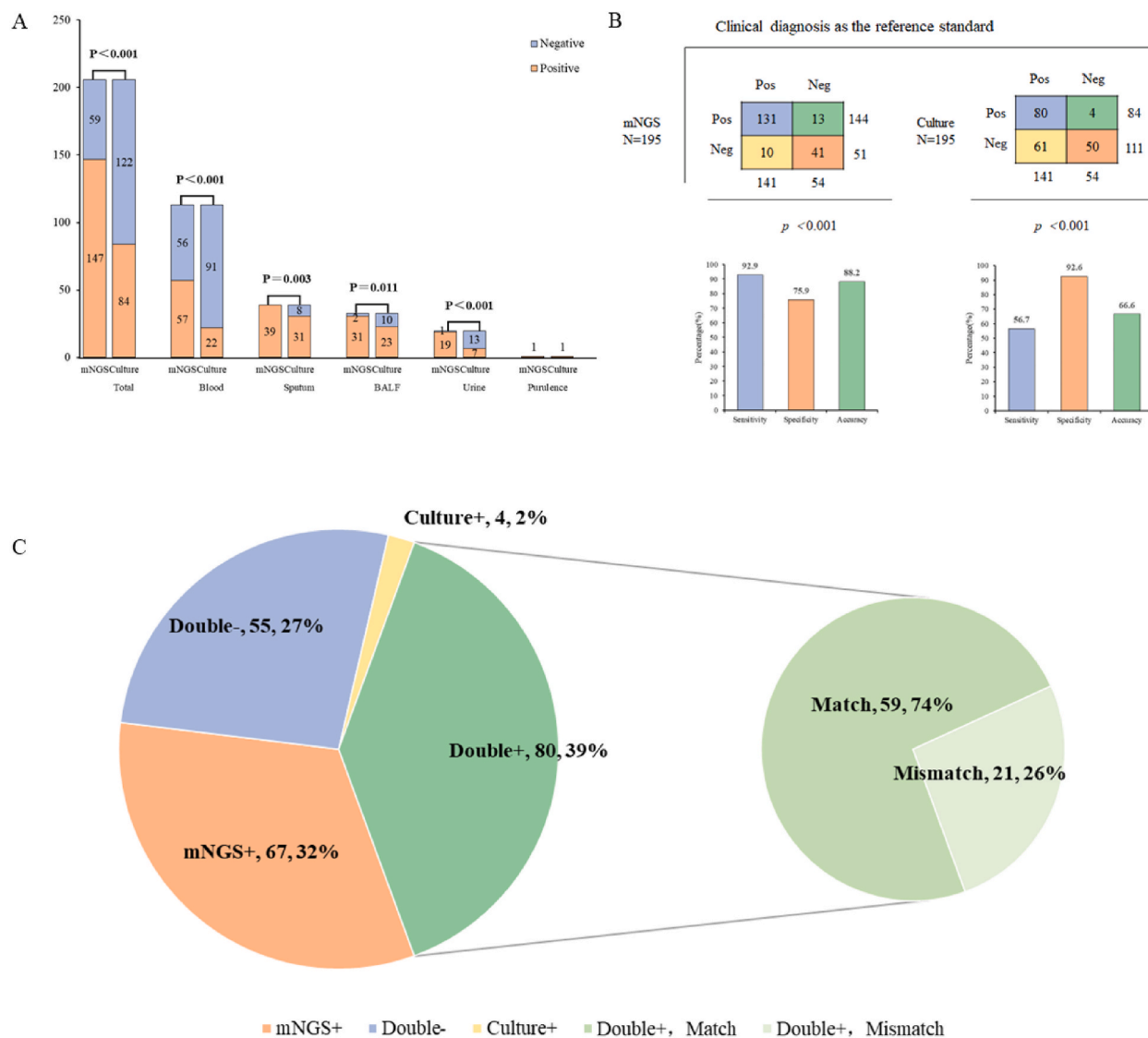
**Fig. 2.** Clinical Diagnostic Effect of mNGS and Culture. (A)The positivity of disparate sample types between mNGS and microbial culture, (B) The sensitivity, specificity and accuracy of mNGS and culture, (C) Comparison of detection consistency. *P* value lower than 0.05 was deemed as statistically significant.

the identification of negative samples, achieving a specificity of 75.9 % versus 92.6 % for culture methods.

Among all the detected samples, 80 (39 %) yielded positive results for both mNGS and culture, while 55 (27 %) were deemed double negative (Fig. 3). mNGS was able to detect pathogens in 67 (32 %) samples, whereas culture was only able to detect pathogens in 4 (2 %) samples. Notably, mNGS successfully identified pathogens in 67 (32 %) of the samples, whereas culture was only able to detect pathogens in 4 (2 %) of the specimens. A further comparison of the double-positive samples indicated that 59 samples (74 %) exhibited consistent pathogen detection, while the results for 21 cases (26 %) were found to be mismatched (Fig. 2C).

### 3.3. Clinical Diagnostic Effect of mNGS and culture in the detection for bacteria, fungi and viruses

The analysis comprised a total of 141 samples after removing samples that did not match the infection sites and submitted locations as determined by clinical diagnosis. A comprehensive analysis revealed the presence of 63 distinct pathogens. Among the bacterial species identified, the most frequently detected were *Klebsiella pneumoniae* (21/141,14.9 %), *Escherichia coli* (20/141,14.2 %), *Staphylococcus aureus* (18/141,12.8 %), *Streptococcus pneumoniae* (16/141,11.3 %), and *Enterococcus faecium* (15/141,10.6 %). These five species had detection frequencies above 10 occurrences. The three predominant fungal species identified were *Candida albicans* (31/141,22.0 %), *Pneumocystis jirovecii*(13/141,9.2 %), and *Candida glabrata*(10/141,7.1 %). In viruses, *human cytomegalovirus* was detected only 11 times by mNGS. The detection of different species by the two methods was shown in Fig. 3. Out of the eight bacteria
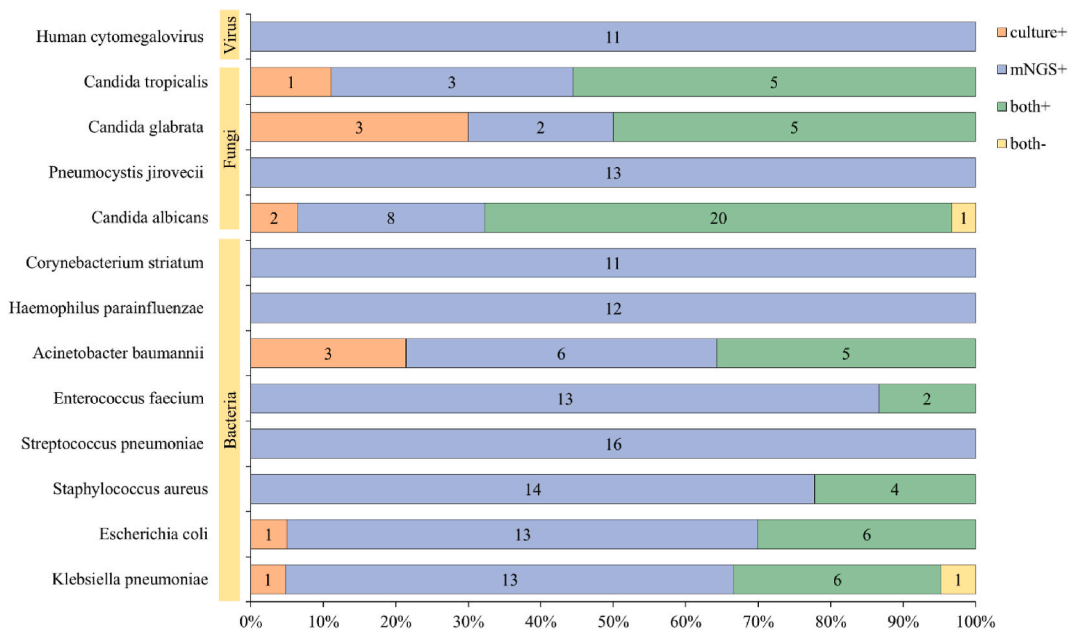
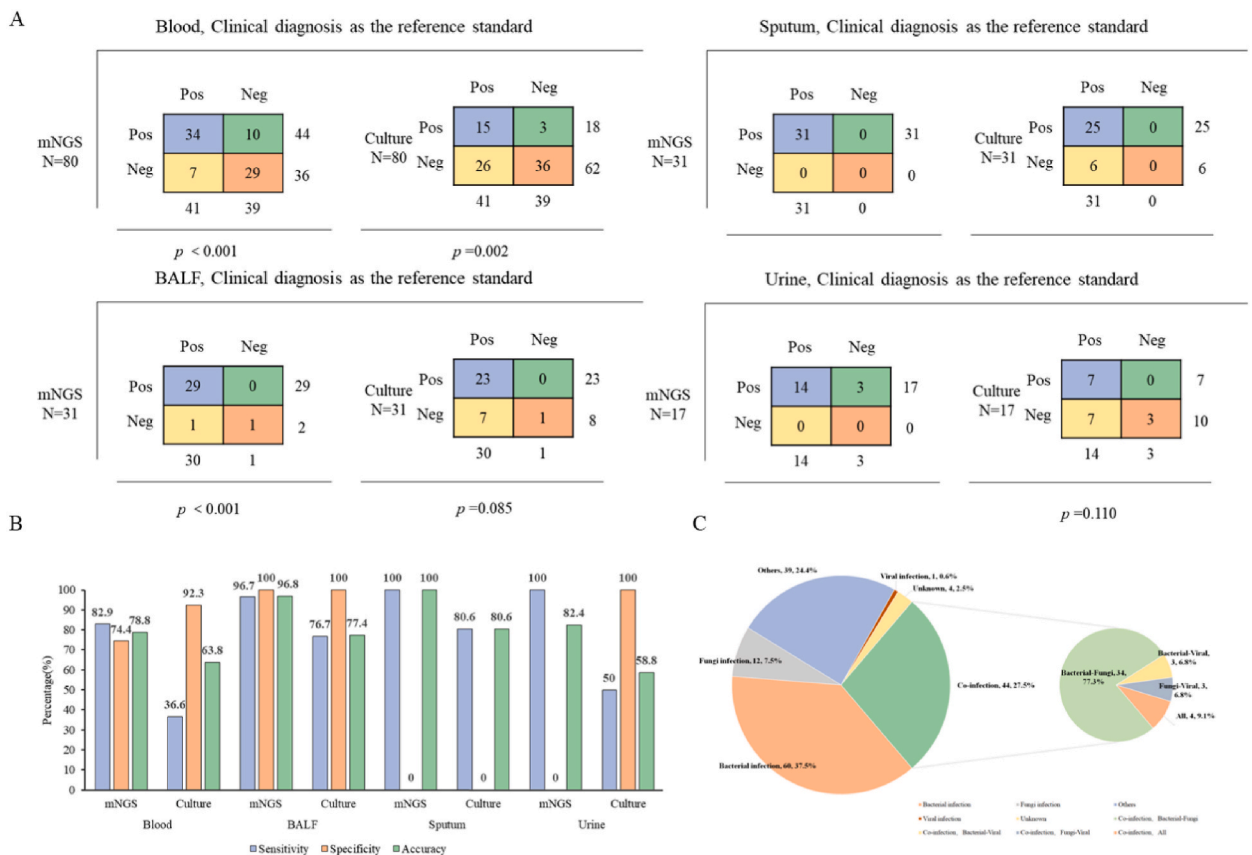**Fig. 3.** The detection of different types of species by the two methods.



**Fig. 4.** Diagnostic performance of mNGS and culture in sepsis. (A) The number of positive cases of different samples, (B)The sensitivity, specificity and accuracy of different samples, (C) Diagnosis of infection types.

with the highest detection frequencies, the number of cases that were exclusively positive by mNGS exceeded those that were solely positive by culture. Notably, *Corynebacterium striatum, Haemophilus parainfluenzae,* and *Streptococcus pneumoniae* were exclusively identified using mNGS. In contrast, with regards to fungi, the detection results for fungi from mNGS and culture methods were comparable, with the exception of *Pneumocystis jirovecii,* where detection was only reported via mNGS. Additionally, the number of positive cultures for *Candida glabrata* slightly surpassed that detected by mNGS, with one more instance reported.

### 3.4. Clinical Diagnostic Effect of mNGS and culture in sepsis

As illustrated in Fig. 4B, the diagnostic performance for infections varies across different sample types in a cohort of 160 sepsis samples. Based on the ultimate clinical diagnosis serving as the reference standard, the diagnostic accuracy of mNGS in blood, bronchoalveolar lavage fluid, sputum, and urine were 78.8 %, 96.8 %, 100.0 %, and 82.4 %, respectively (note that one case of purulence was excluded from the calculation). In contrast, the accuracy of culture was 63.8 % for blood, 77.4 % for bronchoalveolar lavage fluid, 80.6 % for sputum, and 58.8 % for urine. The data further indicate that the sensitivity of mNGS generally exceeds that of culture. However, in blood and urine samples, the sensitivity of mNGS was lower compared to the sensitivity of culture. Fig. 4A presents the incidence of positive results for both mNGS and culture methods, along with clinical diagnoses across various samples.

The most prevalent infection type was bacterial infections, accounting for 60 cases (37.5 %). This was followed by mixed infections, which were observed in 44 cases (27.5 %). Additionally, fungal infections were identified in 12 instances (7.5 %), while viral infections were detected in only one case (0.6 %). It is noteworthy that among the 39 cases (24.4 %), a majority were identified as infections in
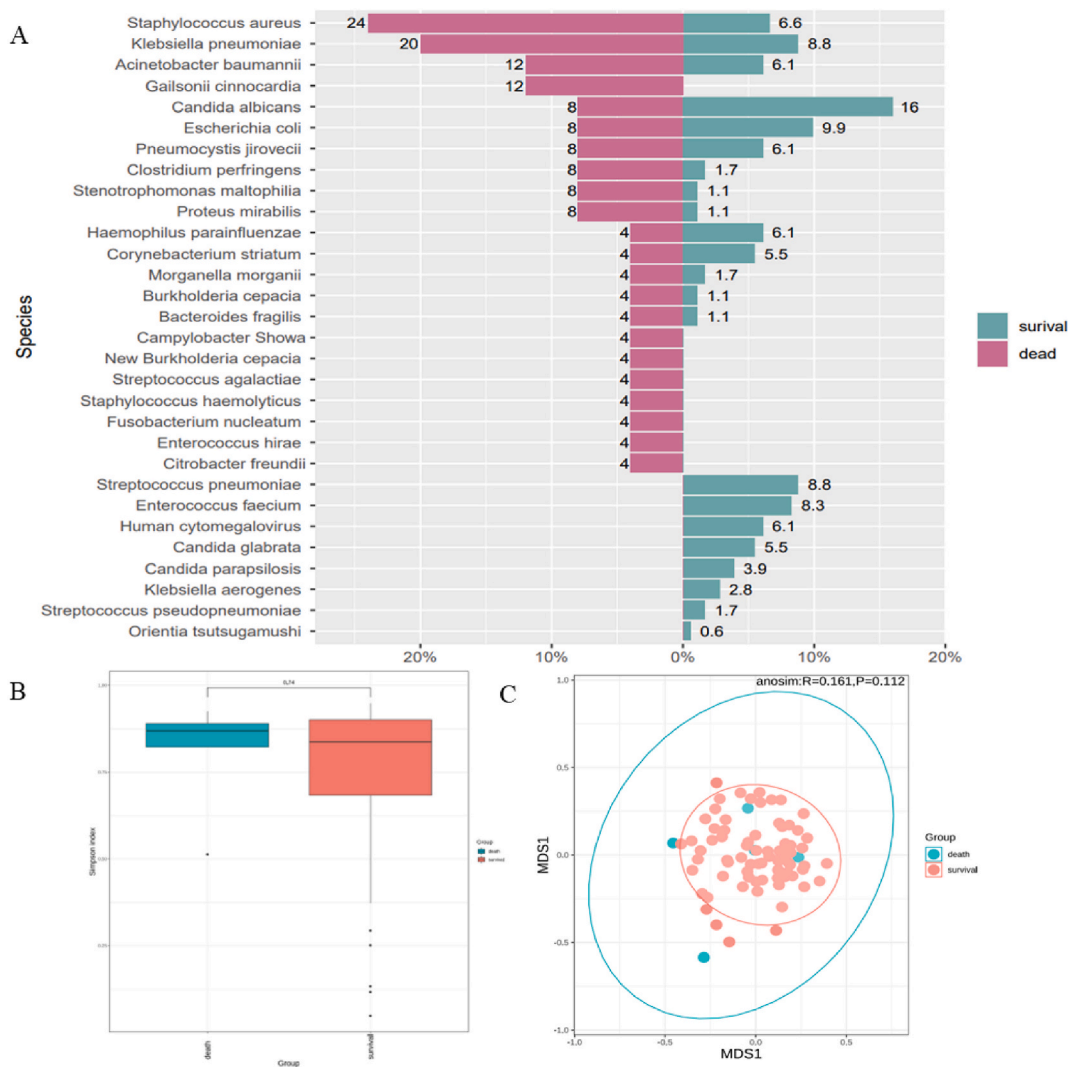


**Fig. 5.** Microbial communities in blood of patients with different outcomes (A) The main pathogen distribution of death and survival group, (B) Simpson index reveals the alpha diversity within the samples, (C) Beta diversity of the groups.

other regions such as the abdomen, urinary tract, or lungs. The collected samples were all non-infected sites, resulting in the inability to identify the pathogen. In terms of mixed infections, bacterial-fungal infections exhibited the highest frequency of detection, occurring in 34 instances and representing 77.3 % of the total. Additionally, there were three cases (6.8 %) of bacterial-virus and fungal-virus co-infections, while four cases (9.1 %) involved the presence of three different types of pathogens (Fig. 4C).

*3.5. The microbial composition and the correlation between Sofa score and antibiotic use in patients with different prognosis*

According to the prognosis, all patients were separated into two groups: survival and death. Comparing the frequency of pathogen detection between the two groups revealed that the top five species detected were different. In the death group, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli*, and *Pneumocystis jirovecii* were the most prevalent species pathogens. In the survival group, the most prevalent species pathogens were *Candida albicans, Streptococcus pneumoniae, Enterococcus faecium, Escherichia coli,* and *Haemophilus parainfluenzae* (Fig. 5A). Despite the differences in the most prevalent pathogens, there were similarities in the species observed in both groups, which led us to conduct a more in-depth analysis of the microbial communities based on blood samples. The analysis of alpha and beta diversity, as depicted in the figure, indicated that there was no statistically significant distinction in the microbiota's richness and diversity between two groups (Simpson index: $p = 0.74$, NMDS: $p = 0.112$) (Fig. 5B and C).

Sperman correlation analysis was performed to explore the correlation between clinical indicators and microbial, such as age, SOFA score, inflammatory markers after admission, and whether antibiotics were used within 1 h. The top 20 species ranked by abundance are presented in Fig. 6. *Candidatus Planktophila sulfonica* was negatively and distinctly correlated with white blood count. *Acinetobacter johnsonii* was positively correlated with SOFA score, and *Acinetobacter junii* of the genus *acinetobacter* was positively correlated with two indicators both age and white blood count. In addition, *Pseudomonas aeruginosa* was also positively correlated with age. Except for *Candidatus Planktophila sulfonica*, the other three species could cause bloodstream infection.
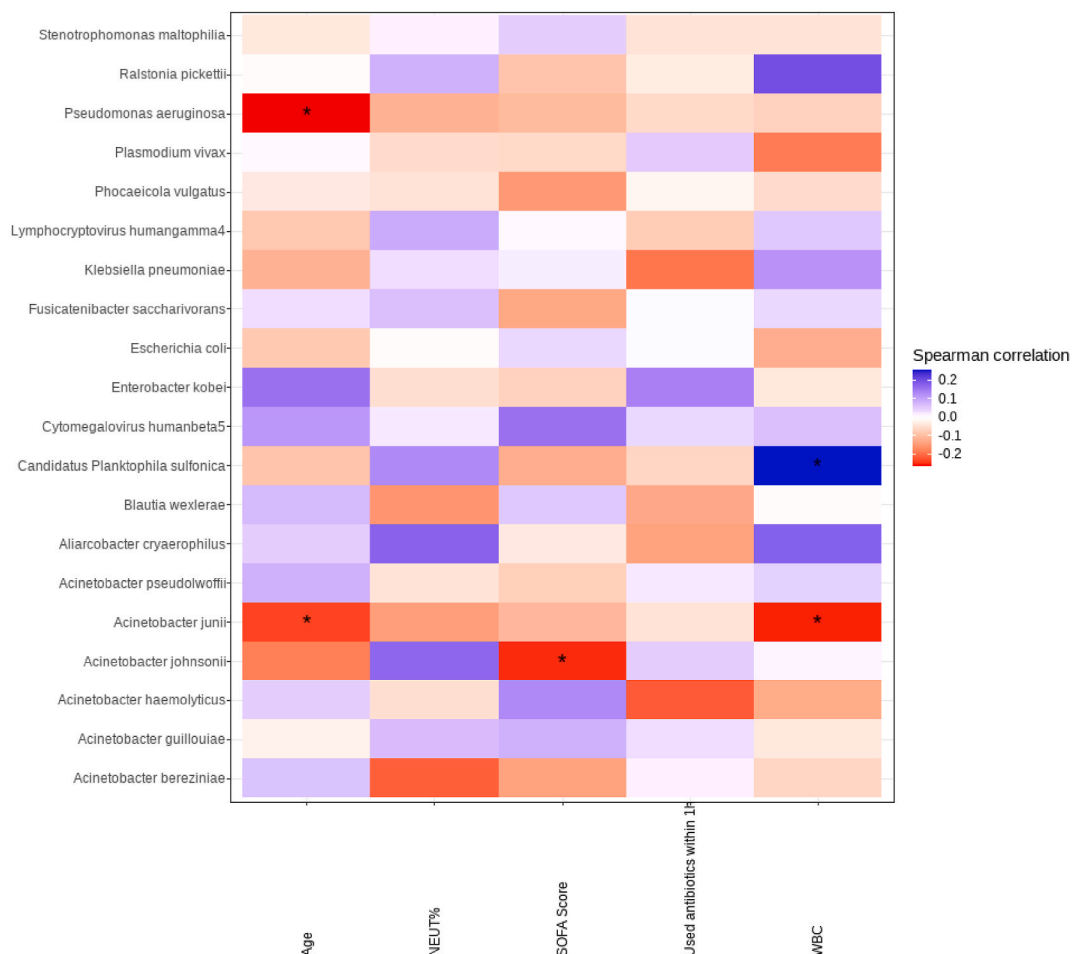


Fig. 6. Clinical and microbial correlation analysis. NEUT, Neutrophil count; WBC, White Blood Count; The symbol * represent significance p < 0.05.

## 4. Discussion

Currently, both the incidence of infectious diseases and difficult pathogen infections are increasing progressively. Timely identification of the causative pathogens is crucial, as delayed diagnosis can lead to the progression of the disease and potentially escalate to life-threatening sepsis accompanied by multiple organ failure. Traditional in-hospital detection methods, such as blood cultures, urine cultures, sputum cultures, and serological antigen assays, often exhibit low positive rates. Additionally, these methods typically yield results that take considerable time to process and can pose challenges in diagnosing uncommon pathogens. As a second-generation sequencing technology, mNGS offers a rapid and precise diagnostic instrument for etiologically diagnosing clinical infectious pathogens.

In this study, we investigated the diagnostic performance of mNGS and in-hospital detection techniques in patients with emergent infection. According to the baseline data, most patients had additional underlying conditions, primarily cardiovascular disorders, malignancies, and immunosuppression. In the event of infection, the median SOFA score was higher than 4 points, and the white blood cell count, absolute neutrophil count, C-reactive protein, and procalcitonin were all considerably higher than normal values. Moreover, hypertension was identified as an independent risk factor for 28-day mortality in infected patients requiring mechanical ventilation in the ICU [9]. The mortality rate in this study was 9.8 %. A majority of the patients exhibit a medical background characterized including hypertension, diabetes, or the administration of vasoactive medications, invasive ventilator-assisted breathing, and hemodialysis. The median length of hospital stay was 12 days. The aforementioned findings show that the majority of the infected research participants had other underlying disorders and were in critical condition. Because the median SOFA score met the diagnostic standards for sepsis, patients with severe illness and urgency may have been more likely to undergo mNGS detection.

In most of previous investigations, the positive detection rate of mNGS pathogens was significantly higher than that of blood culture [7,10,11]. The overall diagnostic sensitivity of sepsis patients in ICU increased considerably from 12.82 % using blood culture alone to 30.77 % using mNGS alone [12]. In this study, the positive rate of culture was 40.8 %, which could be increased to 71.4 % by using mNGS ($p < 0.001$). The another study analyzed 96 blood culture results and 48 mNGS results from 48 sepsis patients, revealing positivity rates of 62.5 % for mNGS compared to only 14.5 % for blood cultures, with these results showing significant disparity ($p < 0.001$) [13]. In our study, cultures yielded a positive rate of 19.5 %, while mNGS demonstrated a positive rate of 50.5 %. mNGS also exhibited significantly higher positive rates in sputum, bronchoalveolar lavage fluid, and urine samples. The sensitivity of mNGS was 92.9 %, the accuracy was 88.2 %, and the specificity of culture was 92.6 %. In comparing the two detection methods, 39 % of cases were positive for both mNGS and blood culture, with 74 % of the identified pathogens being consistent between the two methods. The low sensitivity of culture maybe related to the culture conditions of pathogens and the possibility of introducing contamination. Meanwhile, the detection of skin symbiotic bacteria is also worth considering. Further analysis of the discrepancy between the two detection methods revealed that *Staphylococcus hominis* was identified in 75 % of the four positive culture samples, and it was a part of the common bacterium found on human skin. In the mNGS results, *Staphylococcus hominis* was initially identified as a suspect background microorganism, making it challenging to determine the clinical diagnosis and management for the affected patients. Therefore, the detection of *Staphylococcus hominis* blood culture results were attributed to the possibility of sample contamination, which infrequently caused systemic infection and also reflected the limitations of blood culture technology in comparison to mNGS technology. The main advantage of mNGS is that it can detect bacteria, fungi, virus and other atypical pathogens at one time based on a large pathogen database. And even in environments with effective antibiotic treatment, mNGS has a higher positive detection rate [14, 15].

Bacteria constitute a significant group of pathogens responsible for a wide array of illnesses. Clinically significant bacteria are responsible for approximately 7.7 million fatalities annually, accounting for 56.2 % of deaths associated with sepsis [2]. This study found that mNGS had superior performance in bacterial detection and demonstrated clear advantages in identifying *Pneumocystis jirovecii*. Moreover, *Candida* species were recognized as the significant causative agent of sepsis [16,17]. The positive outcomes associated with both diagnostic approaches reveal noteworthy similarities. The presence of a thicker cell wall in comparison to bacteria creates significant challenges in achieving complete nucleic acid release. While mNGS has gained significant maturity and widespread adoption in clinical diagnosis, traditional methods remain essential for complementing mNGS in the diagnosis of complicated illnesses.

In 160 samples of sepsis patients, we compared the diagnostic performance of mNGS and culture in different sample types Our results indicated that, contrary to conventional expectations, the samples exhibiting the highest sensitivity, specificity, and accuracy were not blood, but rather urine and bronchoalveolar lavage fluid. This finding confirms that lung infections are the predominant cause of sepsis, accounting for more than 50 % of all cases, followed by abdominal and urinary tract infection [18,19]. This trend is consistent with findings from Li's study on immunocompromised patients [20]. Among the four sample types analyzed, the sensitivity of culture methods was found to be lower than that of mNGS, potentially due to prior antibiotic administration. Sepsis is a critical systemic condition, wherein the timely initiation of antibiotics is crucial for effective early intervention. Prompt initiation of anti-infective therapy should occur within 1 h of the onset of severe infection [21]. Currently, the etiological foundations of such infections are often unclear, underscoring the importance of advanced diagnostic methods like mNGS.

In the microbial analysis of patients with different prognosis outcomes based on blood samples, it was found that the microbial α and β diversity of the death group and the survival group were different, but there was no significant difference. It is hypothesized that the observed phenomenon is associated with the sample size. Among a total of 113 blood samples, a majority of 100 samples were observed in the survival group, while a minority of just 13 samples were found in the death group. Furthermore, *Stenotrophomonas maltophilia* and *Acinetobacter guillouiae* were identified as distinct species. These species are characterized by their low pathogenicity and generally do not cause significant harm to individuals with normal immune function. However, there has been a noticeable rise in

mortality rates attributed to sepsis induced by *Stenotrophomonas maltophilia* in recent years [22]. Additionally, *Acinetobacter guillouiae* has been identified as another pathogen capable of causing severe consequences [23]. The microorganisms in question exhibit resistance to many antibiotics, hence caution should be exercised in employing empirical treatment strategies.

In this investigation, it was determined that *Staphylococcus aureus, Klebsiella pneumoniae*, and *Acinetobacter baumannii* were the most common pathogens in the death group. A meta-analysis conducted in 2022, encompassing 341 studies with a total of 536,791 patients, revealed that the estimated seven-day mortality rate for *Staphylococcus aureus* infections was 10.4 %, the one-month mortality rate was 18.1 %, and the one-year mortality rate was 30.2 % starting from 2011 [24]. The total mortality rate among patients infected with *carbapenem-resistant Klebsiella pneumoniae* was 42.14 %, whereas the overall mortality rate among patients infected with *carbapenem-sensitive Klebsiella pneumoniae* was 21.16 % [25]. Therefore, early detection of pathogens with a high mortality rate in clinical practice using mNGS is crucial in situations where in-hospital detection methods have a long detection window and low accuracy. Early detection of high mortality pathogens in clinical practice through mNGS is essential due to the lengthy time limit and low accuracy of in-hospital detection methods, as well as the complexity and diversity of infection sites and pathogens of patients. While identifying pathogens, mNGS can also detect drug resistance genes. Despite drug resistance genes and drug sensitivity phenotypes are not completely consistent, it can still provide auxiliary diagnostic information for clinical practice in order to reduce the mortality of infection. In the analysis of the correlation with clinical indicators, it was found that *Acinetobacter johnsonii, Acinetobacter junii*, and *Pseudomonas aeruginosa* caused severe bloodstream infections [26–28], which was consistent with most sepsis patients in our cohort. The detection of *Candidatus Planktophila sulfonica* made us outside, which is a plant virus [29,30], did not find pathogenic information. In addition, for special organisms, such as *Pneumocystis jirovecii, Mycobacterium tuberculosis, Nocardia, Clostridium perfringens*, etc., mNGS can rapidly provide etiological evidence despite being negative in hospital culture.

This study also has several limitations. Firstly, the number of samples collected for this retrospective analysis was insufficient. Not all patients had both mNGS and in-hospital etiological results, which could introduce selection bias and potentially influence the outcomes of the statistical analyses. Additionally, no further research was conducted to assess the consistency of findings across multiple samples provided by patients who contributed more than one specimen. Furthermore, due to the limited number of samples, many pathogens were excluded from the analysis when comparing the quantitative determination of different pathogen sequences between surviving and deceased patients. Consequently, the potential value of quantitative determinations of various pathogen sequences in the diagnosis of sepsis was not thoroughly explored.

## 5. Conclusion

In the early diagnosis of patients with acute infections, mNGS has a higher positive rate than in-hospital routine culture, which can determine the pathogen of infection rapidly and accurately. Moreover, mNGS can analyze clinical indicators and patient prognoses by leveraging the vast amounts of data generated.

## Ethics and consent

The studies involving human participants were reviewed and approved by IEC for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University (No.2023-503). Written informed consent was collected from each patient upon sample collection according to the protocols approved.

## Funding

## Data availability statement

The original contributions presented in the study are included in the article material. The pathogen reads of our study were deposited in the Genome Warehouse in the National Genomics Data Center under project PRJCA028169.

## CRediT authorship contribution statement

**Lingyu Wei:** Writing – original draft, Data curation, Conceptualization. **Jieyu Luo:** Data curation. **Weiwei Wu:** Visualization, Formal analysis. **Jia Yin:** Visualization, Formal analysis. **Zaiyuan Sun:** Data curation. **Xue Xu:** Formal analysis. **Wenqian Gong:** Data curation. **Jia Xu:** Writing – review & editing, Project administration, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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