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ORIGINAL RESEARCH

Metagenomic Next-Generation Sequencing Unveils Prognostic Microbial Synergism and Guides Precision Therapy in Candidemia: A Retrospective Cohort Study

Yuhui Chen[1,2], Meng Li[1,2], Xinai Gan[1,2], Yutong Wang[1,2], Xinzhu Tang[1,2], Yongzhao Zhou[3,4], Ting Niu[1,5,6]

[1]Department of Hematology, Institute of Hematology, West China Hospital, Sichuan University, Chengdu, People's Republic of China; [2]West China School of Medicine, Sichuan University, Chengdu, People's Republic of China; [3]Integrated Care Management Center, West China Hospital, Sichuan University, Chengdu, People's Republic of China; [4]Department of Pulmonary and Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, People's Republic of China; [5]State Key Laboratory of Biotherapy, Collaborative Innovation Center of Biotherapy, West China Hospital, Sichuan University, Chengdu, People's Republic of China; [6]National Facility for Translational Medicine (Sichuan), West China Hospital, Sichuan University, Chengdu, People's Republic of China

Correspondence: Ting Niu, Department of Hematology, Institute of Hematology, West China Hospital, Sichuan University, No. 37 GuoXueXiang Street, Chengdu, Sichuan Province, 610041, People's Republic of China, Email niuting@wchscu.cn; Yongzhao Zhou, Integrated Care Management Center, West China Hospital, Sichuan University, No. 37 GuoXueXiang Street, Chengdu, Sichuan Province, 610041, People's Republic of China, Email yongzhaozhou001@wchscu.cn

Purpose: Candidemia remains a life-threatening infection, compounded by diagnostic delays and limited prognostic tools. While metagenomic next-generation sequencing (mNGS) offers rapid pathogen detection, its prognostic utility and therapeutic impact in candidemia remain unestablished.

Patients and Methods: This retrospective cohort study analyzed 97 candidemia patients with positive blood mNGS at West China Hospital (2020–2024). Multivariable logistic regression and survival analyses identified mortality predictors, while therapeutic impacts were assessed through antifungal regimen modifications.

Results: The 28-day mortality was 44.3% (43/97). Blood mNGS outperformed cultures in species identification (5 vs 4 species) and co-infection detection. Bacterial co-detections (HR=2.00, 95% CI:1.15–3.48; $p<0.05$) doubled mortality risk. SOFA score was the strongest mortality predictor (adjusted OR=1.29 per point; $p<0.001$). mNGS-guided antifungal initiation reduced mortality by 52.4% in treatment-naïve patients (22.6% vs 75.0%; $p<0.05$), though regimen adjustments in pretreated cases showed no benefit ($p>0.05$). Notably, *Candida* species exhibited equivalent virulence (log-rank $p>0.05$), and mNGS read counts lacked prognostic value ($p>0.05$).

Conclusion: mNGS transforms candidemia management by enabling early risk stratification (via SOFA scores and co-infection profiles) and precision therapy initiation. Its capacity to unmask high-risk bacterial synergists and guide time-sensitive interventions supports integration into diagnostic algorithms, particularly for culture-negative cases. Further validation of standardized mNGS protocols is warranted to maximize clinical impact.

Keywords: candidemia, metagenomic next-generation sequencing, mNGS, prognostic model, microbial synergism, precision therapy

Introduction

Candidemia and invasive candidiasis are one of the most prevalent healthcare-associated invasive fungal diseases.[1] Despite advances in antifungal therapy, these infections carry substantial mortality rates ranging from 25% to 50%,[2] with recent multinational cohort studies reporting 90-day mortality as high as 43%.[3] The diagnostic challenges inherent to candidemia exacerbate this clinical burden - blood cultures, while considered the diagnostic gold standard,[4] demonstrate suboptimal sensitivity (50–75% overall sensitivity) and require prolonged incubation times (median 2–5 days).[5,6] The 1,3-β-D-glucan (BDG), though widely adopted, lacks *Candida* specificity and shows substantial false-positive rates in

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patients receiving hemodialysis or albumin transfusions.[7] Emerging biomarkers like T2Candida® show promise but require specialized equipment and carry significant per-test costs.[8] Molecular techniques such as quantitative fluorescent polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR), when applied to *Candida* detection, are limited to single-target nucleic acid detection, resulting in low analytical efficiency.[9,10] The first 12–48 hours post-infection constitute a critical therapeutic window for candidemia management, as delayed antimicrobial intervention is strongly associated with elevated mortality rates.[11] Consequently, broad-spectrum empirical antifungal therapy is commonly administered to high-risk patient populations. However, this practice may lead to increased healthcare costs and treatment-related adverse effects.

Metagenomic next-generation sequencing (mNGS) has revolutionized infectious disease diagnostics through its hypothesis-free pathogen detection capability.[12] This technology enables simultaneous identification of >10,000 pathogens from 0.2 mL plasma within 24 hours.[12] Many studies have demonstrated the superior diagnostic value of mNGS in infectious diseases.[13,14] This technology demonstrates superior sensitivity (84.78% for invasive pulmonary fungal infections)[15] and exceptional capability in identifying rare pathogens compared to conventional methods.[16] Notably, mNGS excels in detecting polymicrobial co-infections.[17]

Current mortality predictors for candidemia (central venous catheters, parenteral nutrition, broad-spectrum antibiotic exposure) derive predominantly from culture-dependent studies.[18,19] However, the prognostic significance of mNGS-detected *Candida* DNAemia remains unexplored, particularly in culture-negative cases. This study aimed to (1) identify 28-day mortality (a common endpoint in critical care and infectious disease research) predictors specific to mNGS-confirmed candidemia, (2) evaluate the clinical utility of mNGS through survival analysis, and (3) quantify the real-world impact of mNGS results on antifungal therapy optimization. By correlating pathogen load dynamics with clinical outcomes, we sought to establish mNGS-driven prognostic biomarkers and redefine its role in candidemia management.

Methods

Study Design

This retrospective cohort study was conducted at West China Hospital between September 2020 and January 2024 (Figure 1). We consecutively enrolled adult patients (≥18 years) who underwent blood mNGS testing at the Precision Medicine Center and met the following criteria: (1) positive detection of *Candida* species by blood mNGS; (2) availability of complete clinical data; (3) hospitalization during the testing period. For patients with multiple positive

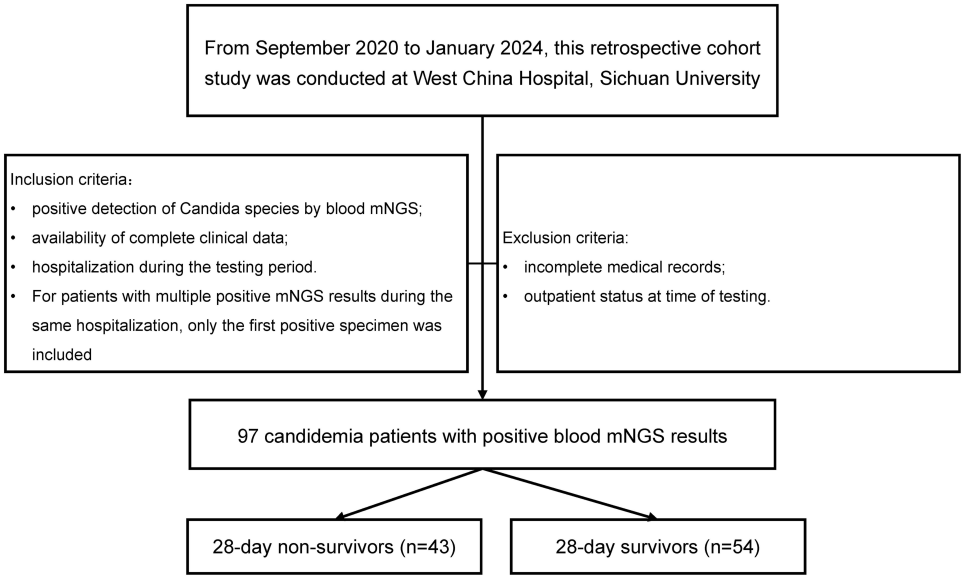

Figure 1 Flowchart of patient enrollment.

mNGS results during the same hospitalization, only the first positive specimen was included. Exclusion criteria included: (1) incomplete medical records; (2) outpatient status at time of testing.

To minimize selection bias, all consecutive eligible cases were systematically screened using predefined criteria. The primary objective was to identify factors associated with 28-day mortality and perform survival analysis in Candidemia patients detected by blood mNGS. Secondary objectives included evaluating the clinical impact of mNGS results on therapeutic decision-making and patient prognosis.

Data Collection

Clinical data were systematically extracted from multiple sources including: Hospital Information System (HIS), Electronic Medical Records (EMR), Laboratory Information System (LIS), Bedside monitoring systems. Data collection was performed using a standardized, pre-designed Excel template capturing: demographic characteristics, comorbidities, recent invasive procedures (within 30 days), immunosuppressive therapy history, baseline laboratory parameters, concomitant microbiological results (blood cultures and other fungal diagnostics within ±7 days of mNGS testing), Sequential Organ Failure Assessment (SOFA) scores, vital signs and organ support requirements, antifungal therapy regimens (pre- and post-mNGS testing) and so on.

Ethical Statement

The study protocol was approved by the Ethics Committee of West China Hospital, Sichuan University (Approval No. 2023–890). Informed consent was waived due to the retrospective design. All data were anonymized to protect patient confidentiality.

Metagenomic Next-Generation Sequencing Detection

The patients' plasma samples were collected and sent to the Precision Medicine Key Laboratory of Sichuan Province and Precision Medicine Center for testing. DNA extraction was performed using the TIAN amp Micro DNA Kit (DP316, TIANGENBIOTECH, Beijing, China). Quality control of the libraries was conducted using the Agilent 2100 Bioanalyzer, targeting fragment sizes of 200–300 bp. The concentration of the DNA libraries was measured using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Inc). High-throughput sequencing was performed on the MGISEQ-2000 platform (MGI, China) using a PE100 (100-bp paired-end) strategy. An average of 20 million high-quality reads per sample were generated, providing sufficient depth for microbial detection. The raw sequencing data were first processed using Fastp (version 0.20.0) to remove low-quality reads (reads with >10% N bases or >50% of bases with quality score <5), adapter sequences, and short reads (<50 bp). High-throughput mNGS sequencing was performed using the MGI2000 platform. The raw sequencing data were processed to remove low-quality and contaminant sequences. The filtered data were aligned to the human reference genome using BWA (http://biobwa.sourceforge.net/). The remaining sequences were compared against the BGI Microbial Reference Database (PMDB), which includes 6350 bacterial, 1064 fungal, 4945 viral, and 234 parasitic species.[12] The average coverage for key pathogens (eg, *Candida* species) was calculated using SAMtools and was approximately 20x, ensuring confident identification. To exclude contamination and implausible results, the following criteria were applied:[20] (1) Species with reads per million (RPM) < 3 times the RPM in negative control samples; (2) Common known contaminant species; (3) Species with < 3 sequences at the species level; (4) Species with uneven distribution compared to the reference genome, indicating potential random fragments rather than true sequences; (5) Common respiratory tract bacteria reported in the literature; (6) Species with strong positive detections in other samples in the same batch (sequence number > 10,000 or much higher than other samples), considering the sample extraction order to exclude contamination; (7) Common environmental species that are not pathogenic. For bacterial species, the top 10 genera were ranked by sequence number, and only the top 2 species within each genus were considered, except for highly pathogenic species such as *Klebsiella pneumoniae*, which were retained if they met the exclusion criteria. Fungi, viruses, and parasites were retained if they met the exclusion criteria, with an additional requirement for parasites to have a sequence number > 10 due to their larger genome size and higher similarity to the human reference genome.

Candida Blood Culture

Blood samples from patients with suspected candidemia were collected and inoculated into aerobic and anaerobic blood culture bottles (BD BACTEC™). Bottles were incubated in an automated system (BACTEC FX) at 35°C for up to 5 days. Positive bottles with yeast cells on Gram stain were subcultured on Sabouraud Dextrose Agar and CHROMagar™ Candida. Pure isolates were primarily identified using MALDI-TOF MS (Bruker Biotyper). Ambiguous results were confirmed with auxiliary tests, such as the germ tube test or biochemical panels (API 20C AUX).

Statistical Analysis

All analyses were conducted in R (version 4.4.1) with key packages including mice for multiple imputation, glmnet for LASSO regression, autoReg for regression modeling, and tableone for baseline table generation. GraphPad Prism (version 8) was used for Kaplan-Meier curve generation and visualization. Continuous variables were assessed for normality using Shapiro–Wilk tests, with normally distributed data presented as mean±SD and non-normal data as median[IQR]. Categorical variables were reported as counts (percentages). Missing data were imputed via chained equations (mice package, 10 iterations, predictive mean matching). Least absolute shrinkage and selection operator (LASSO) regression with log(λ) penalty was implemented using 10-fold cross-validation (cv.glmnet function). The optimal lambda (λ=0.021) minimizing binomial deviance retained 7 predictors. Univariable and multivariable logistic regression analyses were performed using autoReg package's autoReg() function with default stepwise selection (AIC criterion). Continuous variables were z-score standardized prior to modeling. The package automatically generated formatted regression tables including ORs, 95% CIs, and p-values. Survival analysis was performed with the time origin defined as the date of initial positive mNGS detection. The primary endpoint was mortality within a 12-week follow-up period, with surviving patients right-censored at the earlier of either 12 weeks post-testing or last documented clinical contact. Statistical significance was defined as two-tailed $p<0.05$ unless otherwise specified.

Results

Study Population Characteristics

A retrospective cohort of 97 candidemia patients with positive blood mNGS results was analyzed (September 2020-January 2024), with 28-day mortality reaching 44.3% (43/97). As detailed in Table 1, non-survivors exhibited distinct

Table 1 Baseline Characteristics

	Overall, N=97	**Non-Survivors, N=43**	**Survivors, N=54**	***P*-value**
Sex (%)				**0.039**
Male	60 (61.9)	32 (74.4)	28 (51.9)	
Female	37 (38.1)	11 (25.6)	26 (48.1)	
Age (years), (mean (SD))	56.20 (19.68)	64.26 (19.05)	49.78 (17.86)	**<0.001**
The reads detected for *Candida* by mNGS (median [IQR])	10.00 [3.00, 93.00]	7.00 [2.50, 41.00]	12.50 [3.50, 100.50]	0.177
mNGS simultaneously detected bacteria (%)	59 (60.8)	27 (50.0)	32 (74.4)	**0.025**
mNGS simultaneously detected fungi (%)	15 (15.5)	7 (13.0)	8 (18.6)	0.631
mNGS simultaneously detected virus (%)	79 (81.4)	41 (75.9)	38 (88.4)	0.192
Diagnosis (%)				**0.004**
Infection	82 (84.5)	42 (97.7)	40 (74.1)	
Colonization	15 (15.5)	1 (2.3)	14 (25.9)	

(*Continued*)

Table 1 (Continued).

	Overall, N=97	Non-Survivors, N=43	Survivors, N=54	*P*-value
EORTC/MSGERC dignosis (%)				0.452
Proven	26 (26.8)	12 (27.9)	14 (25.9)	
Probable	43 (44.3)	22 (51.2)	21 (38.9)	
Likely	24 (24.7)	8 (18.6)	16 (29.6)	
Unlikely	4 (4.1)	1 (2.3)	3 (5.6)	
Hemotologic malignancy or solid tumor (%)	27 (27.8)	12 (27.9)	15 (27.8)	1
Immune-mediated inflammatory disease (%)	7 (7.2)	2 (4.7)	5 (9.3)	0.634
Respiratory diseases (%)	6 (6.2)	3 (7.0)	3 (5.6)	1
Hepatobiliary disease (%)	9 (9.3)	2 (4.7)	7 (13.0)	0.294
Pancreatits (%)	6 (6.2)	1 (2.3)	5 (9.3)	0.325
Trauma (%)	6 (6.2)	1 (2.3)	5 (9.3)	0.325
Surgery (%)	30 (30.9)	10 (23.3)	20 (37.0)	0.216
Abdominal surgery (%)	20 (20.6)	8 (18.6)	12 (22.2)	0.853
Immunosuppressant (%)	11 (11.3)	5 (11.6)	6 (11.1)	1
Corticosteroids (%)	8 (8.3)	3 (7.1)	5 (9.3)	1
Chemotherapy or radiation therapy (%)	18 (18.6)	9 (20.9)	9 (16.7)	0.784
Diabetes mellitus (%)	25 (25.8)	13 (30.2)	12 (22.2)	0.508
Hypertension (%)	32 (33.0)	16 (37.2)	16 (29.6)	0.568
CKD (%)	10 (10.3)	4 (9.3)	6 (11.1)	1
Dialysis (%)	29 (29.9)	18 (41.9)	11 (20.4)	**0.038**
Chronic lung diseases (%)	6 (6.2)	4 (9.3)	2 (3.7)	0.476
Chronic heart insufficiency (%)	18 (18.6)	9 (20.9)	9 (16.7)	0.784
Drinking (%)	10 (10.3)	4 (9.3)	6 (11.1)	1
Smoke (%)	19 (19.6)	7 (16.3)	12 (22.2)	0.635
TPN (%)	34 (35.1)	15 (34.9)	19 (35.2)	1
Neutropenia (%)	13 (13.4)	4 (9.3)	9 (16.7)	0.449
Hospitalization (median [IQR])	30.00 [14.00, 55.00]	19.00 [9.00, 34.50]	42.00 [21.50, 76.50]	**<0.001**
SOFA score (median [IQR])	10.00 [5.00, 15.00]	14.00 [11.00, 18.00]	6.00 [4.00, 9.75]	**<0.001**
CVC/PICC removal (%)				**0.004**
None	20 (20.6)	17 (31.5)	3 (7.0)	
Yes	37 (38.1)	21 (38.9)	16 (37.2)	
No	40 (41.2)	16 (29.6)	24 (55.8)	
Exposure to antibiotics (%)	68 (70.1)	35 (81.4)	33 (61.1)	0.052
ICU admission (%)	52 (53.6)	29 (67.4)	23 (42.6)	**0.026**

(*Continued*)

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Table 1 (Continued).

	Overall, N=97	Non-Survivors, N=43	Survivors, N=54	*P*-value
Respiratory support (%)				**0.002**
Invasive Mechanical Ventilation	42 (43.3)	27 (62.8)	15 (27.8)	
Nasal Cannula or Mask Oxygen Therapy	46 (47.4)	12 (27.9)	34 (63.0)	
Non-invasive Ventilation	7 (7.2)	4 (9.3)	3 (5.6)	
Non-oxygenated	2 (2.1)	0 (0.0)	2 (3.7)	
BDG (ng/L), (median [IQR])	101.72 [37.00, 229.48]	140.30 [55.47, 340.83]	37.03 [37.00, 140.07]	**0.003**
GM test (pg/mL), (median [IQR])	0.12 [0.06, 0.24]	0.16 [0.08, 0.56]	0.11 [0.05, 0.14]	**0.004**
TBIL (umol/L), (median [IQR])	14.90 [9.00, 39.80]	17.90 [10.90, 54.85]	12.70 [8.67, 24.62]	0.113
ALT (IU/L), (median [IQR])	22.00 [11.00, 42.00]	20.00 [12.00, 39.50]	23.00 [11.00, 42.00]	0.925
AST (IU/L), (median [IQR])	29.00 [18.00, 53.00]	37.00 [22.00, 54.50]	23.00 [17.25, 48.50]	0.085
ALB (g/L), (median [IQR])	32.30 [28.40, 36.00]	30.70 [28.10, 34.90]	34.15 [30.38, 36.93]	**0.031**
GLB (g/L), (median [IQR])	24.10 [19.90, 30.90]	22.30 [19.90, 28.25]	24.50 [20.02, 32.18]	0.158
Scr (umol/L), (median [IQR])	72.00 [50.00, 120.00]	96.00 [64.00, 143.50]	60.00 [46.00, 95.75]	**0.003**
eGFR (mL/min/1.73m^2), (median [IQR])	89.95 [47.99, 114.51]	61.28 [40.23, 96.72]	107.56 [71.94, 122.91]	**0.001**
LDH (IU/L), (median [IQR])	296.00 [207.00, 412.00]	362.00 [255.50, 492.00]	238.00 [183.00, 309.25]	**<0.001**
BNP (ng/L), (median [IQR])	649.00 [100.00, 2426.00]	1723.00 [544.00, 4751.00]	251.00 [100.00, 1094.25]	**0.001**
Hb (g/L), (median [IQR])	84.00 [71.00, 94.00]	82.00 [70.00, 90.00]	84.50 [74.00, 101.75]	0.095
PLT (×10^9/L), (median [IQR])	77.00 [36.00, 184.00]	55.00 [37.00, 101.50]	146.00 [35.25, 213.50]	**0.014**
WBC (×10^9/L), (median [IQR])	7.47 [4.19, 12.05]	10.98 [4.67, 16.65]	5.47 [4.06, 10.62]	**0.008**
Neutrophil percentage (median [IQR])	80.70 [70.40, 88.80]	88.20 [76.45, 92.95]	75.00 [69.23, 83.30]	**<0.001**
Lymphocytes (×10^9/L), (median [IQR])	0.62 [0.32, 1.12]	0.50 [0.32, 1.02]	0.64 [0.33, 1.15]	0.336
Neutrophils (×10^9/L), (median [IQR])	6.30 [2.82, 10.40]	9.82 [3.64, 15.32]	4.35 [2.65, 7.62]	**0.003**
PCT (ng/mL), (median [IQR])	1.36 [0.30, 3.26]	2.34 [1.02, 5.06]	0.37 [0.17, 1.82]	**<0.001**
CRP (mg/L), (median [IQR])	80.10 [33.10, 159.00]	123.00 [56.65, 191.50]	60.05 [26.62, 127.25]	**0.004**
IL-6 (pg/mL), (median [IQR])	77.90 [27.20, 388.00]	182.00 [52.65, 548.50]	44.30 [18.73, 106.00]	**0.003**

Note: Bold values indicate statistical significance ($p < 0.05$).

Abbreviations: mNGS, Metagenomic next-generation sequencing; SD, Standard deviation; IQR, Interquartile range; SOFA, Sequential Organ Failure Assessment; PCT, Procalcitonin; CRP, C-reactive protein; CVC/PICC, Central venous catheter/Peripherally inserted central catheter; BDG, 1,3-β-D-glucan; GM, Galactomannan; TPN, Total parenteral nutrition; LDH, Lactate dehydrogenase; ALB, Albumin; GLB, Globulin; TBIL, Total bilirubin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Scr, Serum creatinine; eGFR, Estimated glomerular filtration rate.

clinical profiles: advanced age (64.3±19.0 vs 49.8±17.9 years, p<0.001), male predominance (74.4% vs 51.9%, p=0.039), and elevated disease severity markers including SOFA scores (median 14 vs 6, p<0.001) and PCT levels (median 2.34 vs 0.37 ng/mL, p<0.001). Critical care interventions were more frequent in non-survivors, with 62.8% requiring mechanical ventilation versus 27.8% in survivors (p=0.002). Paradoxically, bacterial co-detection rates were lower in non-survivors (50.0% vs 74.4%, p=0.025), while viral co-detection showed comparable prevalence (75.9% vs 88.4%, p=0.192).

Microbial Co-Detection Patterns and Diagnostic Superiority of Blood mNGS in Candidemia

Blood mNGS revealed distinct mortality-associated microbial co-detection profiles (Figure 2A): *Klebsiella pneumoniae* (22/97, 22.7%) and *Acinetobacter baumannii* (21/97, 21.6%) were the predominant bacteria, with *K. pneumoniae* detection linked to higher mortality (68.2% vs 31.8% survivors), while *Pseudomonas aeruginosa* (3/3) exclusively occurred in survivors. Fungal co-detections, including *Aspergillus* spp. (9/97, 77.8% mortality), *Pneumocystis jirovecii* (2/97, 100% mortality) and *Mucor* (2/97, 100% mortality) strongly predicted poor outcomes, as did viral co-detections of Epstein-Barr virus (58.3% mortality) and *HHV-1* (83.3% fatality). Conversely, Torque teno virus (TTV) favored survivors (75.0% vs 25.0%). Co-infection type stratification (Figure 2B) showed "Bacteria+Virus" combinations exclusively in non-survivors (7.0% vs 0%), whereas "virus-only" infections predominated in survivors (33.3% vs 16.3%). Blood mNGS outperformed culture in Candida speciation (Figure 2C and D), detecting 5 species versus culture's 4, with 5-fold higher *Candida albicans* (41 vs 3 cases) and unique identification of *Candida glabrata* (7/97) and rare *Candida intermedia* (3/97), while culture predominantly isolated *Candida parapsilosis* (53.3%, 8/15).

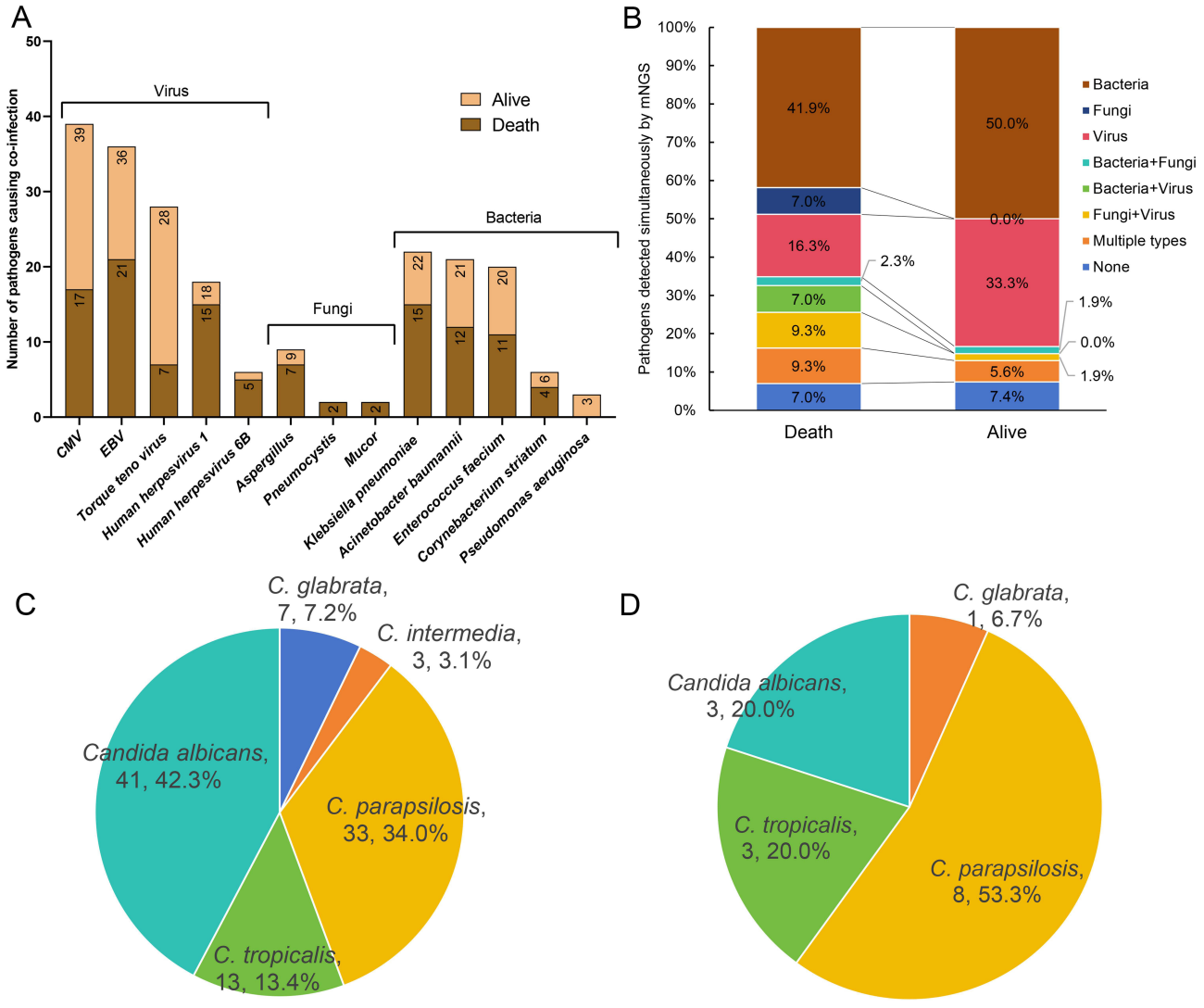

Figure 2 Microbial Co-detection Profiles and Candida Species Distribution by Blood mNGS versus Blood Culture. (**A**) Pathogen distribution in survivors versus non-survivors; (**B**) Co-detection types identified by blood mNGS in survivors versus non-survivors; (**C**) *Candida* species distribution detected by blood mNGS; (**D**) Candida species distribution detected by blood culture.

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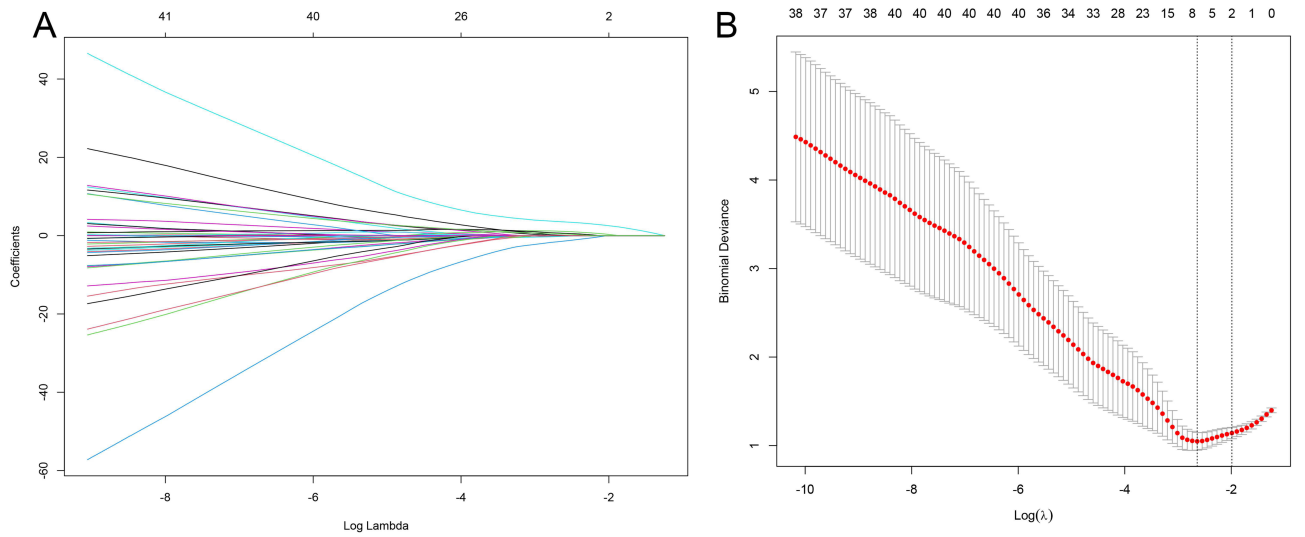

Figure 3 Selection of high-risk independent predictor variables by LASSO regression. (**A**) LASSO Coefficient Path Plot. (**B**) Cross-Validated Mean Squared Error for LASSO Model Selection.

Predictive Factors for 28-Day Mortality in Candidemia Patients

Variables associated with 28-day mortality were initially screened through LASSO regression analysis (Figure 3). The regularization path diagram (Figure 3A) demonstrated coefficient trajectories of candidate predictors, while the 10-fold cross-validation curve (Figure 3B) identified optimal lambda values, ultimately selecting seven variables: CVC/PICC removal, hospitalization duration, SOFA score, GM test value, neutrophil count, age, and CRP level. Subsequent univariable and multivariable logistic regression analyses further characterized these predictors (Table 2). The SOFA score emerged as the strongest independent predictor of mortality, demonstrating consistent significance across all analyses (adjusted OR=1.29, 95% CI: 1.14–1.45, p<0.001). Age and hospitalization duration showed borderline significance in the final model (p=0.051 and 0.054, respectively), while CVC/PICC removal exhibited differential effects depending on implementation status. Notably, microbial characteristics revealed by mNGS demonstrated clinical relevance, with GM test values

Table 2 Comparison Between ML-Based Risk Prediction Model of Diagnosis of PJP by mNGS with BALF

Variable	Survivors (N=54)	Non-Survivors (N=43)	Univariable OR (95% CI)	Multivariable OR (95% CI)	Final Model OR (95% CI)
CVC/PICC removal					
None	17 (31.5%)	3 (7%)	Reference	Reference	
Yes	21 (38.9%)	16 (37.2%)	4.32 (1.08–17.32)*	2.31 (0.29–18.53)	
No	16 (29.6%)	24 (55.8%)	8.50 (2.14–33.81)**	3.82 (0.63–23.05)	
Age	49.8 ± 17.9	64.3 ± 19.0	1.04 (1.02–1.07)***	1.03 (1.00–1.06)†	1.03 (1.00–1.06)†
Hospitalization	58.2 ± 55.0	29.5 ± 35.3	0.98 (0.97–1.00)**	0.98 (0.97–1.00)†	0.98 (0.97–1.00)†
SOFA score	6.9 ± 4.8	13.7 ± 4.8	1.31 (1.17–1.45)***	1.25 (1.08–1.44)**	1.29 (1.14–1.45)***
GM test	0.1 ± 0.1	1.0 ± 1.9	8.14 (1.00–65.92)*	1.55 (0.49–4.93)	1.78 (0.51–6.19)
Neut	5.4 ± 4.4	9.6 ± 7.0	1.14 (1.05–1.24)**	1.04 (0.94–1.16)	-
CRP	90.2 ± 97.4	145.7 ± 111.7	1.01 (1.00–1.01)*	1.00 (1.00–1.01)	1.00 (1.00–1.01)

Note: *p<0.05, **p<0.01, ***p<0.001, †Borderline significance (0.05<p<0.10).
Abbreviations: OR, Odds Ratio; CI, Confidence Interval.

showing marginal predictive value in univariable analysis (OR=8.14, p=0.050) that attenuated after adjustment. Traditional inflammatory markers (CRP and neutrophil count) failed to maintain statistical significance in multivariable models.

Therapeutic Impact of mNGS-Guided Interventions

The clinical utility of blood mNGS in guiding antifungal decision-making was evaluated through treatment modification analysis (Figure 4A). Among 43 patients without baseline antifungal therapy at mNGS testing, 31 (72.1%) initiated antifungal agents post-testing (Adjustment group: 25 echinocandins, 5 triazoles, 1 polyene), while 12 (27.9%) remained untreated (Non-adjustment group). The adjustment group demonstrated significantly lower 28-day mortality compared to non-adjustment counterparts (22.6% vs 75.0%, p<0.05) (Figure 4B). In contrast, among 54 patients already receiving antifungals at testing (44 echinocandins, 9 triazoles, 1 polyene), therapeutic regimen modifications based on mNGS results (16/54, 29.6%) did not significantly alter mortality outcomes compared to unchanged regimens (43.8% vs 55.3%, p>0.05) (Figure 4C).

Survival Patterns by Microbial Characteristics

Survival analysis revealed distinct prognostic patterns based on microbial profiles (Figure 5A). No significant survival differences were observed among patients with *C. albicans* (n=41), *C. parapsilosis* (n=33), or other Candida species (n=23) (log-rank p>0.05). However, bacterial co-infected patients (n=59) demonstrated substantially worse survival compared to those without bacterial co-detection (n=38) (HR=2.00, 95% CI: 1.15–3.48, p<0.05) (Figure 5B). These findings highlight the importance of early and accurate identification of microbial pathogens in candidemia, as bacterial co-infections were associated with significantly worse outcomes, suggesting that mNGS may facilitate timely and targeted therapeutic interventions to improve patient prognosis.

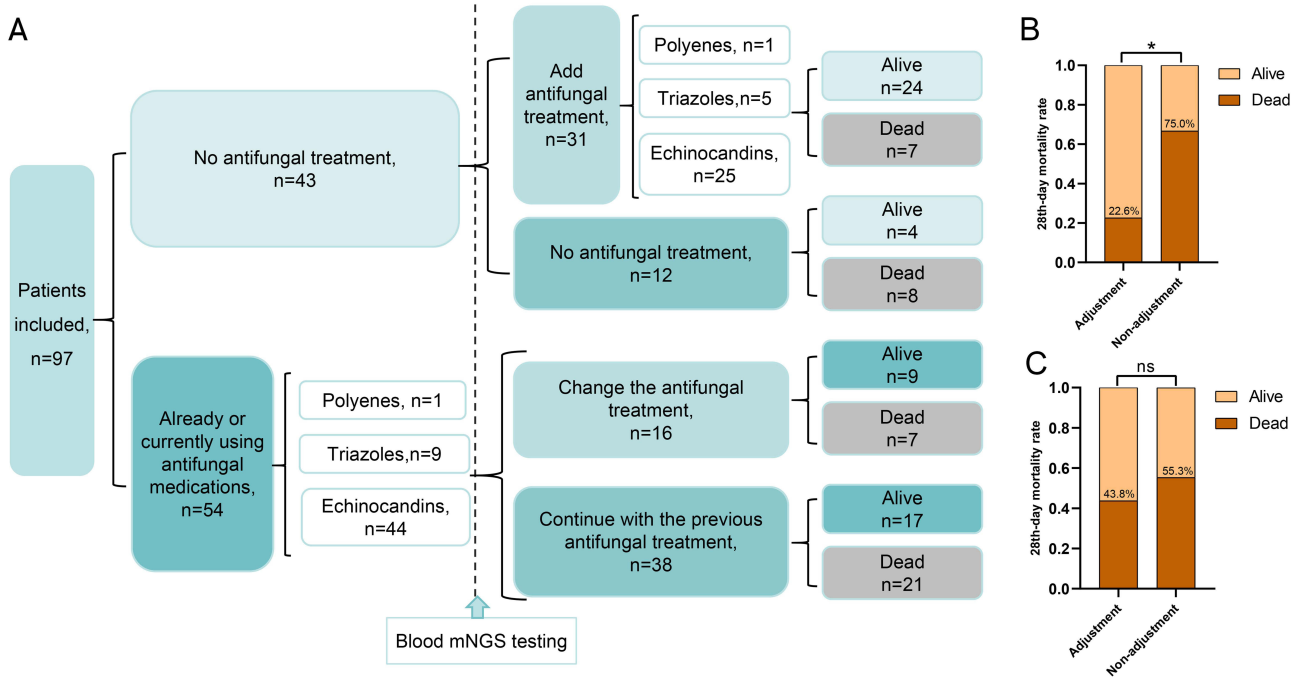

Figure 4 Therapeutic Impact of mNGS-Guided Antifungal Interventions in Candidemia Patients. (**A**) Treatment modification flowchart stratified by baseline antifungal status; (**B**) Comparison of 28-day mortality between mNGS-guided adjustment group (22.6%) and non-adjustment group (75.0%) in treatment-naïve patients (p<0.05); (**C**) Comparison of 28-day mortality between mNGS-guided adjustment group (43.8%) and non-adjustment group (55.3%) in pretreated patients; *p<0.05, ns: not significant.

https://doi.org/10.2147/IDR.S543151

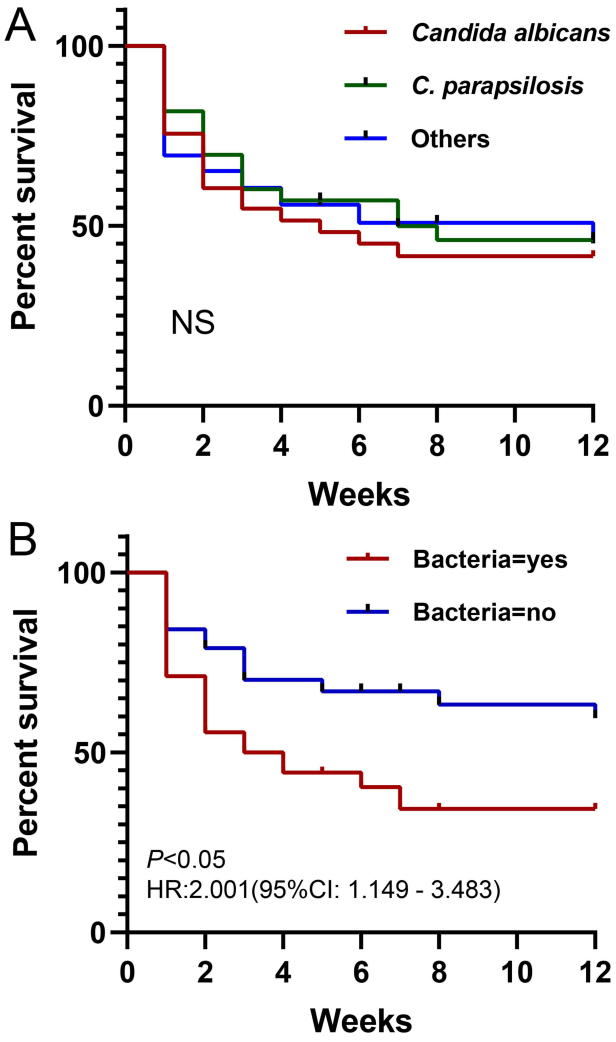

Figure 5 Survival Patterns Stratified by Microbial Characteristics. (**A**) Equivalent virulence across Candida species (log-rank P>0.05); (**B**) Bacterial co-detections doubled mortality risk (HR=2.001, 95% CI:1.149–3.483).

Discussion

This study provides novel insights into the prognostic determinants and clinical utility of mNGS in candidemia management. Notably, our cohort demonstrated a 28-day crude mortality rate of 44.3%, which is lower than previous reports of 60.2–71.1% in ICU-based candidemia populations.[21,22] This discrepancy may reflect mNGS's ability to detect *Candida* DNAemia at earlier stages (including potential colonization) or in less critically ill patients who were excluded from culture-dependent studies due to negative blood cultures. Our findings reveal that mNGS not only enhances diagnostic accuracy for *Candida* species and co-infections but also identifies critical mortality predictors and informs therapeutic strategies, thereby bridging critical gaps in current candidemia management paradigms.

The superior diagnostic performance of mNGS over conventional blood cultures aligns with prior studies, as evidenced by its ability to detect a broader range of *Candida* species (*C. glabrata, C. intermedia*) and polymicrobial co-infections.[23] Notably, mNGS identified *Candida albicans* (41/97), *C. parapsilosis* (33/97), and *C. tropicalis* as the predominant species, consistent with recent epidemiological trends.[24,25] In contrast, blood cultures (positive in only 15.5% [15/97] of cases) predominantly isolated *C. parapsilosis* (53.3%, 8/15), followed by *C. albicans* and *C. tropicalis*. This disparity highlights mNGS's enhanced sensitivity in overcoming the limitations of blood culture, particularly in patients with low fungal burdens or prior antifungal exposure. The higher mortality observed in patients with bacterial co-detections, particularly *K. pneumoniae* and *A. baumannii* (clinically significant pathogens requiring therapeutic

intervention[26,27]), underscores the clinical relevance of mNGS in unmasking complex infection profiles that drive poor outcomes. It should be noted that the observed exclusive association between *P. aeruginosa* and survival outcomes may be related to the limited sample size in this study, which could introduce potential bias. The strong prognostic implications of fungal (*Aspergillus, Mucor*) and viral (HHV-1, Epstein-Barr virus) co-detections highlight mNGS's unique capacity to identify high-risk co-pathogens that traditional methods often miss, enabling targeted antimicrobial escalation.

The SOFA score emerged as the strongest independent mortality predictor (adjusted OR=1.29 per 1-point increase, p<0.001), emphasizing that underlying organ dysfunction—rather than *Candida* species per se—drives outcomes in mNGS-detected candidemia. This aligns with sepsis pathophysiology where host response outweighs pathogen virulence. While CVC/PICC removal is strongly recommended in guidelines,[28,29] our data revealed suboptimal compliance (37/77, 48.1% removal rate) and paradoxical associations: non-removal was linked to higher mortality in univariable analysis (OR=8.50, 95% CI:2.14–33.81), but this association attenuated after adjusting for SOFA scores (aOR=3.82, 95% CI:0.63–23.05). The attenuated predictive value of traditional biomarkers (CRP, neutrophils) in multivariable models underscores the limitations of inflammatory markers in fungal sepsis and emphasizes the need for pathogen-specific tools like mNGS to refine risk stratification. Furthermore, the number of sequence reads aligned to a specific pathogen reflects technical variables including microbial nucleic acid load, extraction efficiency, and host DNA proportion, rather than representing true pathogen burden.[17,30] Notably, in our study, *Candida*-specific read counts showed no correlation with 28-day mortality (Survivors vs Non-survivors: median [IQR] 7.00 [2.50–41.00] vs 12.50 [3.50–100.50], p> 0.05), with paradoxically higher read counts observed in the survivor group. This discrepancy underscores the limitations of using read counts as a standalone diagnostic metric for clinical prognostication.

Therapeutic implications of mNGS were striking: initiating antifungal therapy based on mNGS results reduced 28-day mortality by 52.4% (75.0% vs 22.6%, p<0.05) in treatment-naïve patients, demonstrating its critical role in enabling timely, species-directed therapy. Conversely, the lack of mortality benefit when adjusting pre-existing regimens suggests either delayed intervention in advanced disease or potential antimicrobial resistance—a hypothesis supported by the high SOFA scores (median 14) in non-survivors. These findings advocate for early mNGS implementation in suspected candidemia, particularly in culture-negative cases, to accelerate appropriate therapy before irreversible organ failure ensues.

Our survival analysis demonstrated comparable clinical outcomes across *C. albicans, C. parapsilosis*, and other *Candida* species groups (log-rank $P > 0.05$), suggesting equivalent virulence potential among these pathogens in the study cohort. The significant survival disadvantage associated with bacterial co-detections (adjusted HR = 2.001, 95% CI 1.149–3.483; p<0.05) corroborates emerging evidence of Candida-bacterial synergism in biofilm formation and immune evasion mechanisms [30]. These findings advocate for mNGS-guided broad-spectrum antimicrobial strategies in critical candidemia cases, challenging conventional antifungal-centric protocols.

Limitations and Future Directions

This study has several limitations. First, its single-center retrospective design introduces potential selection bias, though systematic enrollment mitigated this risk. Second, the lack of standardized mNGS protocols across laboratories necessitates validation of pathogen load thresholds and contamination controls. Third, therapeutic decisions influenced by mNGS were clinician-dependent, precluding causal inferences. Prospective multicenter studies comparing mNGS-guided versus conventional management are needed to confirm mortality benefits. Future research should also explore dynamic mNGS monitoring to quantify treatment response and investigate host-pathogen interactions through transcriptomic integration.

Conclusion

In candidemia, mNGS transcends diagnostic utility by identifying high-risk co-infections, prognostic biomarkers, and actionable therapeutic targets. The technology's ability to reduce mortality through early antifungal initiation, coupled with its prognostic stratification via SOFA scores and microbial profiles, positions it as a transformative tool in sepsis

management. Integrating mNGS into candidemia diagnostic algorithms could redefine precision medicine approaches for this lethal infection.

Data Sharing Statement

The datasets generated and/or analysised during the current study are not publicly available due but are available from the corresponding author Ting Niu on reasonable request.

Ethics Approval and Informed Consent

This study was reviewed and granted the Ethics Committee of West China Hospital, Sichuan University (Approval No. 2023-890). The requirement for informed consent was waived by the Ethics Committee of West China Hospital due to the retrospective study. We confirmed that the data was anonymized or maintained with confidentiality in line with the Declaration of Helsinki.

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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.

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Disclosure

The authors report no conflicts of interest in this work.

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