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ORIGINAL RESEARCH

Microbiological Diagnostic Performance and Clinical Effect of Metagenomic Next-Generation Sequencing for the Detection of Immunocompromised Patients With Community-Acquired Pneumonia

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Objective: Community-acquired pneumonia (CAP) presents a significant public health concern, necessitating timely and precise diagnosis. Metagenomic next-generation sequencing (mNGS) has shown promise as a powerful tool for pathogen identification in infectious diseases. This study aimed to evaluate the diagnostic efficacy and clinical applicability of mNGS for immunocompromised patients with CAP compared to the culture method.

Methods: This study included 168 patients. We used both mNGS and conventional culture methods to identify the pathogen spectrum and evaluate diagnostic performance. Treatment regimens and clinical outcomes were meticulously documented.

Results: The sensitivity of mNGS was greater than that of the culture method across all samples (79.05% vs 16.03%; $p < 0.001$). mNGS identified pathogens missed by culture in 59.52% of patients and detected polymicrobial infections that were not detected by culture in 47.62% of patients. *Streptococcus pneumoniae, Candida albicans*, and *Human herpesvirus 4* at classification level emerged as the predominant pathogens identified in CAP patients through mNGS. When examining the mNGS results between groups, the proportions of immunocompromised patients with bacterial ($p < 0.001$), fungal ($p < 0.001$), viral ($p < 0.05$), and mixed infections ($p < 0.001$) were all significantly higher than those in immunocompetent patients. Treatment adjustments guided by mNGS were observed in 73.21% of patients. Specifically, a beneficial clinical effect was observed in 50.60% (85/168) of patients, treatment confirmation in 22.62% (38/168) of patients, and no clinical benefit in 26.80% (45/168) of patients based on mNGS-guided antibiotic treatment adjustments.

Conclusion: These findings highlight the diagnostic performance of mNGS for identifying pathogens, particularly in immunocompromised patients vulnerable to infections, offering valuable insights for clinical decision-making.

Keywords: diagnostic performance, clinical effect, immunocompromised, community-acquired pneumonia, metagenomic next-generation sequencing

Introduction

Community-acquired pneumonia (CAP) is a clinical syndrome, which present as fever, cough, chest pain and infiltration of alveolar space, resulting from infections acquired from non-healthcare settings.[1,2] It stands as a leading global cause of

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morbidity and mortality, especially in immunocompromised patients.[2] In China, the incidence of CAP is reported to be 713 per 100,000 person-year across all ages.[3] In the US, the age-adjusted incidence of CAP requiring hospital admission is estimated at 240 per 100,000 adults annually, leading to around 1.5 million hospitalizations.[4] The Global Burden of Diseases, Injuries, and Risk Factors Study reported that 336.5 million lower respiratory tract infections occurred globally in 2016, resulting in 32.2 per 100,000 person worldwide.[5] CAP can be caused by a variety of pathogens, complicating its diagnosis and management. For instance, a multicenter prospective study on severe CAP in China identified the five most common pathogens as *Influenza virus, Streptococcus pneumoniae, Enterobacteriaceae, Legionella pneumophila*, and *Mycoplasma pneumonia*.[6] However, the identification of these microorganisms presents significant challenges for diagnosis and therapy guidance. Despite these hurdles, further research and surveillance of CAP are essential due to its prevalence as one of the most common conditions in China and one of the global burden of infectious disease.[7] Due to the difficulty in early pathogen diagnosis, initial therapy for infection is often empirical. Moreover, between 11.0% and 20.3% of patients failed to respond to the initial treatment or exhibit exacerbated symptoms within the first 72 hours of hospitalization.[8] Initial treatment failure often results from pathogen resistance, polymicrobial infections, respiratory viral infections, and the inability of the initial drug selection to cover the pathogens.[9] Therefore, improving the diagnostic rate of CAP should improve therapeutic decision-making.

Conventional culture-based microbiology is considered as the cornerstone of diagnosing microbiological infections.[10] However, the low sensitivity and time-consuming nature of standard culture-based tools limit the identification of microbial pathogens in CAP, posing challenges in meeting clinical requirements. Recently, metagenomic next-generation sequencing (mNGS) has been widely applied in the detection of pathogenic microorganisms in infectious diseases, as a method that detects whole nucleic acids directly from patient samples.[11–13] This method enables the simultaneous detection of bacteria, fungi, viruses, and parasites at multiple sites, including the bloodstream, respiratory, gastrointestinal, and urinary tract.[14–17] Studies indicate that bronchoalveolar lavage fluid (BALF) mNGS is more sensitive than blood and sputum mNGS in detecting pathogens, but blood also has advantages in identifying pathogens of pneumonia, especially for some viruses.[18,19] Compared to the conventional culture, mNGS has the advantage of being less affected by antibiotics, more sensitive and specific, accepting diverse sample types.[20] It plays a crucial role in identifying mixed, uncommon, and novel pathogen infections, particularly in immunocompromised patients.[21] Nevertheless, the diagnostic performance and management of immunocompromised patients with CAP has not been thoroughly assessed.

Therefore, this study aims to compare the diagnostic performance of mNGS and culture methods for pathogen detection, and the most common pathogens between immunocompromised and immunocompetent patients with CAP, and evaluate the impact of mNGS results on the diagnosis and anti-infective regimens of CAP patients with different immune status.

Materials and Methods

Patients and Study Design

From April 2020 to April 2023, this retrospective study enrolled patients suspected of CAP at the Affiliated Hospital of Chengde Medical College, China. The diagnostic criteria for CAP referred to the 2019 guidelines of the Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS).[22] In short, patients were considered for inclusion if they were ≥18 years, abnormal imaging findings, and other signs of pulmonary infection, combined with ≥1 following items: 1) worsening cough; 2) worsening expectoration of sputum, or dyspnea; 3) hemoptysis; 4) pleuritic chest pain; 5) abnormalities on chest auscultation and/or percussion; 6) fever (≥38.0°C). Exclusion criteria were cystic fibrosis, severe bronchiectasis, patients with incomplete clinical and laboratory data, or if the patient was not willing or able to provide sputum and BALF sample, or BALF samples that did not meet the quality standards required for mNGS. The immunocompromised status was defined based on consensus, determined by meeting the following the previous study.[23] Immunocompromised status was defined by conditions such as neutropenia (<1000 neutrophils/μL), chemotherapy within the past 30 days, long-term corticosteroid use (≥0.3 mg/kg/day of prednisone equivalent for ≥3 weeks), and hematologic malignancies. Immunocompetent patients had no history of these conditions.

This study was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Chengde Medical College (approval no. CyFyLL2020262). All procedures followed were in the Helsinki Declaration, and the International Ethical Guidelines for Biomedical Research Involving Human Subjects. The informed consent was obtained from all individuals.

Specimen Collection and Infection Indices Testing

Satisfactory sputum samples, BALF samples, blood samples, endotracheal aspirates (ETA) samples, and pleural fluid samples were collected from patients to identify pathogens. The primary principles guiding sample collection were as follows: 1) Clinicians strictly adhered to sterile protocols. Collection containers should be strictly sterile, and when obtaining specimens from non-sterile sites, the possibility of contamination by normal flora or colonized bacteria in these parts should be reduced as far as possible. 2) All specimens should be sent to the laboratory as soon as possible after collection. The samples were cultured within a 3-day timeframe. Meantime, BALF samples were used for mNGS detection. The culture procedures were conducted following standard protocols as described by Donnelly et al.[24] Various blood routine parameters were evaluated upon hospital admission, encompassing white blood cell count, neutrophil count, serum bilirubin, creatinine, lactic acid, C-reactive protein and procalcitonin.

DNA Extraction, Library Construction, and Sequencing

Each sample of BALF was at least 5 mL, collected in a dry sterile tube for cryopreservation and transported on dry ice to the Shanghai Biotech Co., Ltd (Shanghai, China). Upon receipt of the specimens, BALF DNA was extracted by the HostZEROTM Microbial DNA Kit (D4310, ZYMO RESEARCH) per the manufacturer's instructions. To prepare the library, more than 5 ng of DNA was digested to an appropriate length (200–300 bp). The fragments were then treated to attach a "Da" tail at the 3′ end and phosphorylate the 5′ end. Next, the fragmented DNA was ligated to adapter sequences using DNA ligase. To ensure purity, splice dimers, redundant splices, and residual reagents were removed with purification beads. Finally, all sample DNA libraries with a concentration exceeding 1 nmol/L were mixed and sequenced on an Illumina NextSeq CN500 sequencer using a single-end strategy with a read length of 75 base pairs. To control the sequencing quality and contamination of each sequencing run, we added internal control, negative control and positive control in each run. Internal parameters, derived from Arabidopsis thaliana and provided by the sequencing manufacturers, were specific molecular tags placed in the sample prior to nucleic acid extraction to monitor the entire process and ensure the quality of the workflow. Sterile water, used as a negative control, allowed for the detection of contamination. Positive controls are real clinical samples proven to contain known pathogens.

Bioinformatic Analysis

High-quality sequencing data were filtered with Fastp software (v0.23.1) by the following steps: 1) removing adapter sequences from reads and discarding reads without insert fragments caused by connector self-ligation; 2) removing low quality reads (length < 20 bp) at 3′ end; 3) removing reads that contain over 10% of N; 4) removing sequences shorter than 15 bp after trimming. Seqtk_sdust (v1.3-r106) was used to assess the complexity of each read, and sequences with low complexity (<0.3) were filtered out. The reads were then aligned against human reference (GRCh38/hg38.p12) using Bowtie2 (version 2.3.4.1) to filter out the residual human source reads that could not be removed experimentally. After removing the plasmid sequence and incompletely assembled chromosomal sequences, species with completely assembled genomes and chromosomes were included in the pathogenic microorganism database. After that, map the microbial reads via bwa (v0.7.15) and classify pathogenic bacteria with whole genome alignment by Kraken software. Pathogenic microbial genomic data were downloaded from the NCBI Genome databases (ftp://ftp.ncbi.nlm.nih.gov/genomes/), and PATRIC (https://www.patricbrc.org/) database. Finally, all identified pathogens underwent analysis to determine alignment sequence number, relative abundance, and genome coverage.

Criteria for Identifying mNGS Positive

The microorganisms suspected as colonizers were excluded based on an in-house background database containing microorganisms detected in more than 50% of samples in the laboratory over the past three months. Suspected

background microorganisms were removed. Then, the remaining bacteria (mycobacteria excluded), along with viruses (mycetes excluded) and parasites, underwent subsequent filtration based on the following criteria:

1) Bacteria or virus: coverage rate scored 10-fold greater than that of any other microbes.[25]
2) Fungi: coverage rate scored 5-fold higher than that of other fungus,[26] or supported by clinical culture.[18]
3) *Mycobacterium tuberculosis* (MTB): for BALF samples, reads ≥1 since its DNA is hard to extract and unlikely to be contaminated;[25] *Nontuberculous mycobacteria* (NTM) were defined positive when the reads ≥10 or ranked within the top 10 in the bacteria list.[26]

Clinical Evaluation

The clinical impact of mNGS was delineated into three aspects. The initiation of targeted treatment or treatment de-escalation was deemed to the beneficial clinical effect. Treatment confirmation, involving treatment discontinuation or continuation, was guided by mNGS to validate current anti-infective therapy aligned with the etiologic diagnosis. No clinical benefit indicated the pathogen could not be identified via mNGS.

Statistical Analysis

Graphical representations were generated using R Project (R 4.0.2, R Core Team; https://www.RProject.org). Normally distributed continuous variables were expressed as mean ± standard deviation, while non-normally distributed variables were presented as median and range. Categorical variables were reported as count and percentage, with comparisons performed using the chi-square test or Fisher's exact test. The McNemar test was used for comparisons of the diagnostic performance between two diagnostic methods. p <0.05 indicates that the differences were statistically significant. SPSS software (version 27.0; IBM Corporation, Armonk, NY, USA) was used for the statistical analysis.

Results

Study Design, Demographic and Clinical Characteristics

A total of 168 patients were enrolled in this study and received empiric antibiotic treatment. The 216 samples from those patients were collected to detect pathogen using culture. The majority (167/216, 77.31%) of the samples were sputum. The rest included 21 samples from endotracheal aspiration (9.72%), 18 samples from blood (8.33%), 8 samples from pleural fluid (3.24%), 2 samples from BALF (0.93%) (Figure 1A). Meanwhile, an mNGS method was developed for pathogen detection from 168 BALF samples.

Demographically, 58 (34.52%) were females, while 110 (65.48%) were males, with an average age of 58.76 years (Table 1). Immunocompromised patients accounted for 22.02%. Among them, 86 patients had underlying diseases, predominantly diabetes mellitus (13.70%) and hypertension (23.81%). On admission, the majority of patients displayed symptoms of fever and cough, while others exhibited fatigue, hemoptysis, and chest pain. Empirical antibiotics therapy was administered prior to sample collection and detection analysis.

Comparison of Microbiological Pathogens Identified by mNGS and Culture

The patients'microbiological results using culture are presented in Figure 1A. The 30 samples from 25 patients tested positive. Out of them, 5 patients had positive results from two samples each, with different infection sites. The 14.88% (25/168) cases tested positive using culture, while 73.21% (123/168) tested positive using mNGS ($p < 0.001$) (Figure 1B). Both culture and mNGS contributed to identify 28 and 329 pathogenic microorganisms, respectively (Supplementary Figure 1A and B). Among the pathogens detected by culture, 86% were bacteria and 14% were fungi. mNGS identified bacteria as the predominant pathogens (72%), followed by viruses (14%), fungi (12%), and mycoplasma (2%). Only mNGS detection was positive in 100 patients (59.52%), whereas only culture result was positive in 2 patients (1.19%) (Figure 1C), culture and mNGS were both positive in 23 of 168 (13.69%) patients and both negative in 43 of 168 (25.60%) patients. In double-positive patients, detection results were completely matched in 8 (4.76%) patients and completely discordant 13 (7.74%) patients. The rest (7.74%) were classified as "partly matched", indicating that at

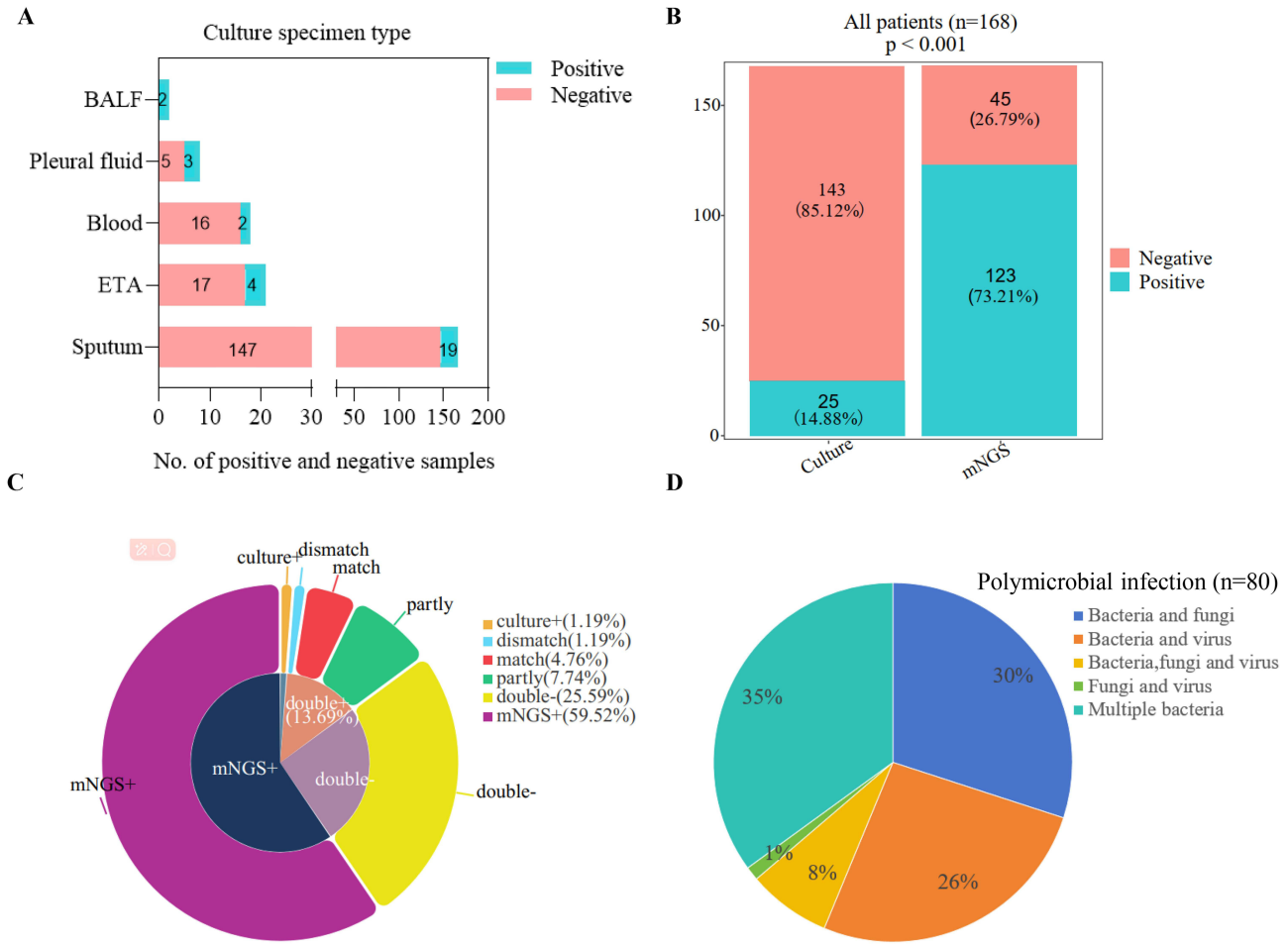

Figure 1 Positive rate and agreement of BALF metagenomic next-generation sequencing (mNGS) compared to culture in 168 CAP patients. (**A**) The composition of sample types and positive sample distribution for culture detection. (**B**) Positive rate comparison for mNGS and culture methods. (**C**) Concordance analysis of pathogens detected via two methods. (**D**) The 80 patients with polymicrobial infections, including 30% patients with bacteria and fungi, 26% patients with bacteria and virus, 35% patients with multiple bacteria, 1% patient with fungi and virus, and 8% patients with three types pathogen infection.

least one but not all, overlapping pathogens were identified in the polymicrobial results. In 47.62% (80/168) patients, mNGS contributed to the identification of polymicrobial infections, detecting multiple bacterial infections in 28 of 80 (35%) patients, bacterial-fungal infections in 24 of 80 (30%) patients, and bacterial-viral infections in 21 of 80 (26%) patients (Figure 1D). Notably, the sensitivity, specificity, positive predictive value and negative predictive value of mNGS were 79.05% (117/148), 90.00% (18/20), 98.32% (117/119) and 36.73% (18/49), respectively (Table 2).

Table 1 Demographic and Clinical Characteristics of the 168 Patients With Community-Acquired Pneumonia (CAP)

Characteristics	All Patients
Age (years, mean±SD)	58.76±15.06
BMI (kg/m^2, mean±SD)	22.47±6.86
Sex (n%)	
Male	110(65.48%)
Female	58(34.52%)

(*Continued*)

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Table 1 (Continued).

Characteristics	All Patients
Smoking history (n%)	
Yes	99(58.93%)
No	69(41.07%)
Drinking history (n%)	
Yes	102(60.71%)
No	66(39.29%)
Immunocompromised (n%)	
Yes	37(22.02%)
No	131(77.98%)
Underlying disease	
Diabetes mellitus	23(13.70%)
Hypertension	40(23.81%)
Chronic bronchitis	5(2.98%)
Chronic obstructive pulmonary disease (COPD)	2(1.19%)
Malignancies	16(9.52%)
Severity	
CURB-65 score	2(0–5)
Laboratory parameters	
White blood cell,10^9/L	8.56(3.5–9.5)
Neutrophil, 10^9/L	72.85(40.0–75.0)
Bilirubin, umol/L	10.30(0.0–26.0)
Serum creatinine, umol/L	58.60(57.0–97.0)
Blood lactic acid, mmol/L	1.30(0.7–2.3)
C-reactive protein, mg/L	36.52(0.05–274.4)
Procalcitonin, ng/mL	0.12(0.00–0.05)

The distribution of pathogens identified by culture and mNGS is shown in Figure 2. The most commonly detected bacteria using only mNGS were *Streptococcus pneumoniae, Haemophilus parainfluenzae* and *Neisseria meningitidis. Mycobacterium, Proteus mirabilis, Streptococcus dysgalactiae*, and *Streptococcus gordonii* were bacteria isolated by only culture. The most detected fungi were *Candida albicans* and *Pneumocystis jirovecii* using mNGS. Additionally, *Human herpesvirus 4* and *Human herpesvirus 7* were the most frequently detected viruses (Figure 2).

Clinical Effects of mNGS Result on Diagnostic Performance and Management in Immunocompromised Patients

As shown in Figure 3, the spectrum of detected pathogens detected by mNGS varied between immunocompromised patients and immunocompetent patients. *Human herpesvirus 6A*, fungi (*Candida tropicalis* and *Candida dubliniensis*) and bacteria

Table 2 Diagnostic Performance of mNGS and Culture Compared With Clinical Final Diagnosis

	mNGS	Culture
Sensitivity	79.05%[a]	16.03%
Specificity	90.00%	100%
PPV	98.32%	100%
NPV	36.73%	25.17%

Notes: p-values were calculated with McNemar-test. [a]mNGS vs culture p<0.001.
Abbreviations: mNGS, metagenomic next-generation sequencing; PPV, positive predictive value; NPV, negative predictive value.

(*Streptococcus tigurinus, Streptococcus agalactiae, Rothia mucilaginosa*, etc.) were only identified in immunocompromised patients. We further compared diagnostic positive rates of mNGS (Figure 4A) between the two groups. Significant differences were observed in the positive rates of bacteria ($p<0.001$), fungi ($p<0.001$), viruses ($p<0.05$), and mixed infections ($p<0.001$) between the two patient groups identified through mNGS analysis. Notably, the proportion of pathogenic microorganisms detected in immunocompromised patients was higher than that in immunocompetent patients ($p<0.05$) (data not shown).

All patients in the study were empirically treated with antibiotics. Based on the mNGS results, 123 patients (73.21%), comprising 93 immunocompetent patients and 30 immunocompromised patients, had their anti-infection treatment strategies adjusted. There was no significant difference observed in treatment adjustment between the two patient groups ($p = 0.221$) (Figure 4B). Among the CAP patients, 50.6% experienced clinically beneficial effects (initiation of targeted treatment and treatment de-escalation), which led to improvement and discharge following mNGS-guided adjustments. Additionally, 22.6% of patients had their treatments confirmed, while 26.8% did not experience clinical benefits (Figure 4C). The beneficial anti-infective adjustments between immunocompromised and immunocompetent patients showed no significant difference (59.46% vs 48.09%; $p = 0.222$) (Figure 4D) (data not shown).

Regarding treatment, the mNGS results led to a direct shift in management for 85 patients who experienced a beneficial clinical effect or to treatment discontinuation for 25 patients who were ultimately discharged based on the mNGS findings. Moreover, 13 cases received a definitive diagnosis that supported the continuation of the prior treatment strategy, while 29 cases did not derive clinical benefit from the mNGS results and required empirical adjustments in antibiotics. It is noteworthy that there were 2 cases of false-positive mNGS results that resulted in incorrect diagnoses, and 1 case received inappropriate antibiotic treatment (Table 3).

Discussion

Our study evaluated the diagnostic performance and clinical impact of mNGS analysis on 168 samples from BALF taken from adult CAP patients. Our findings showed that the sensitivity of mNGS technology was significantly higher than that of culture, demonstrating mNGS as advantageous in diagnosis performance and antimicrobial treatment management.

Although culture is a conventional reference method for assessing diagnostic performance of other tests, its low sensitivity makes it unsuitable in real clinical practice. However, mNGS has potential advantages in terms of speed and sensitivity for detecting pathogens.[14,27] Our findings showed the sensitivity of mNGS was 79.05%, which was much higher than that of culture (16.03%). The result observed by Miao et al is consistent with our data in empirically treated patients (52.7% vs 34.4%).[14] Moreover, the sensitivity of mNGS in our study was higher than that reported by Q. Miao et al, but lower than that observed by Chen et al.[28] This discrepancy may be attributed to differences in patient populations. The high sensitivity of mNGS may be due to the fact that patients with CAP are often initially treated with antimicrobial therapy, which reduces the positive detection rate by culture methods. The sensitivity of mNGS is relatively less affected by prior antibiotics therapy compared to culture.[14]

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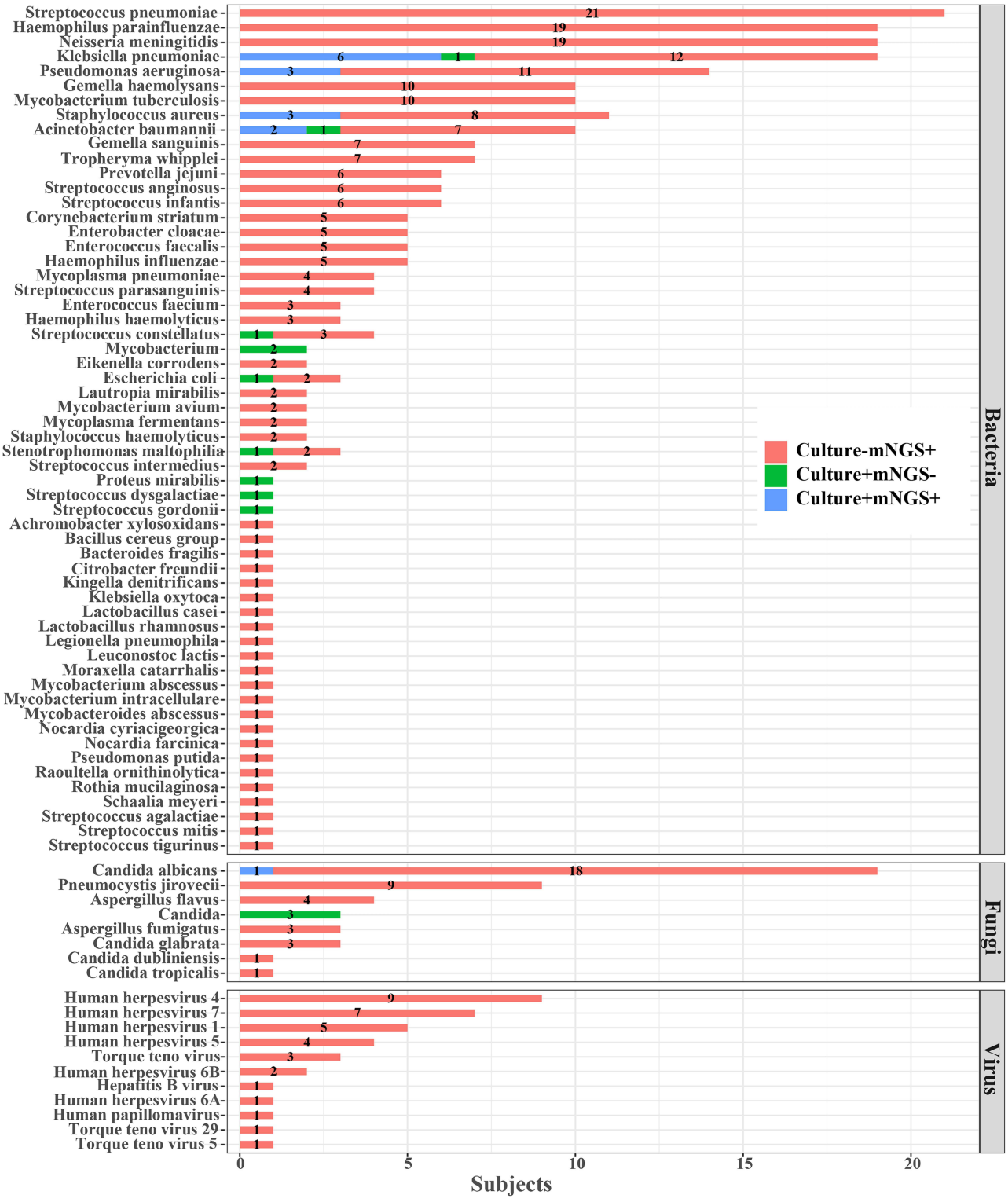

Figure 2 The number of each microbiological pathogen detected by mNGS and culture method. Red bars indicate pathogens detected by mNGS only (culture-mNGS+); Green bars show each pathogen detected by culture only (culture+mNGS-); Blue bars indicate pathogens detected by both mNGS and culture (culture+mNGS+).

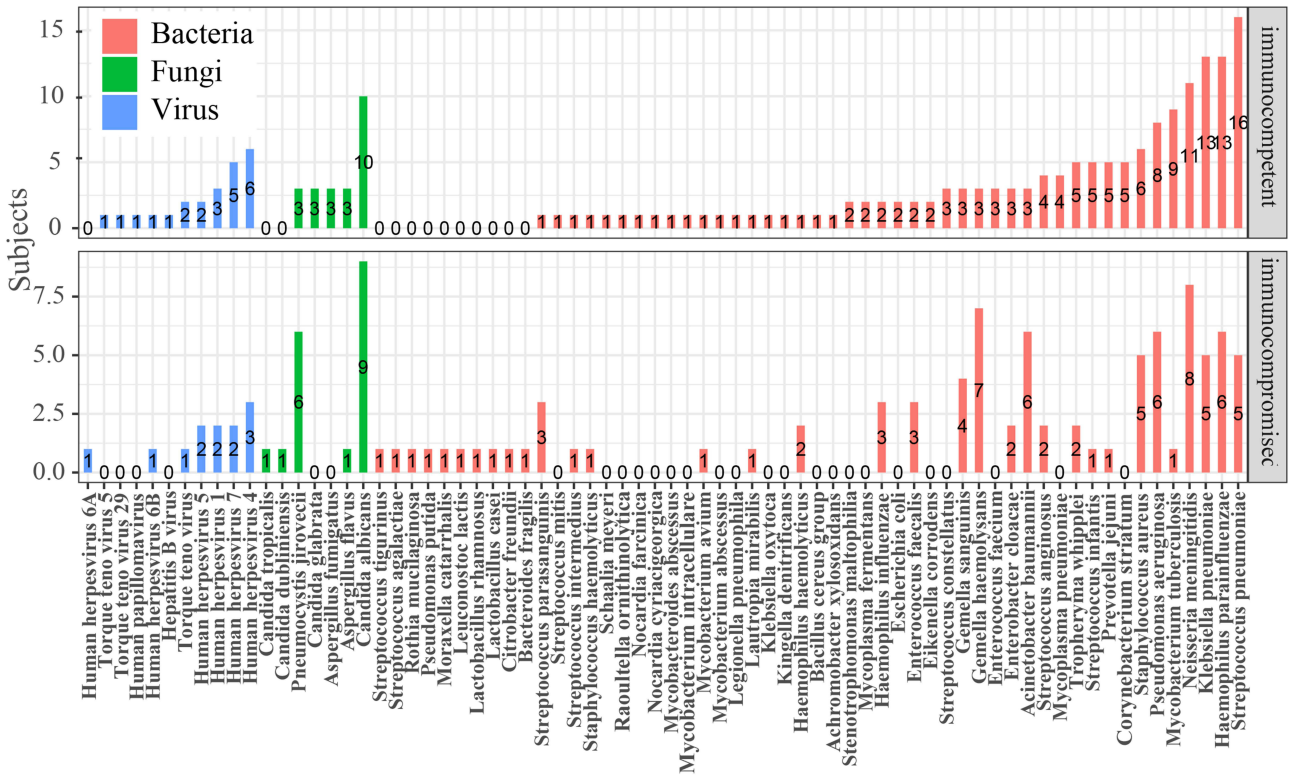

Figure 3 Differences and overlap in the pathogenic spectrum between immunocompromised patients and immunocompetent patients. The detection number of each microbe was calculated. Bacteria were the most detected, followed by viruses and fungi, special pathogens.

Relevant literature shows that the pathogen detection rate of sputum culture ranges from 14% to 21.4%.[29,30] The positive detection rate of BALF mNGS ranged from 66.2% to 100%.[31,32] Our study found that mNGS reported a 73.21% detection rate for pathogens and was obviously higher than that of the culture (14.88%). This is consistent with previous reports comparing total positive rate detected by both mNGS and culture.[33] mNGS proved to be obviously advantageous in detecting pathogens missed by culture in our study. The most commonly detected bacteria included *Streptococcus pneumoniae, Haemophilus parainfluenzae* and *Neisseria meningitidis*, conforming to typical bacteria prevalent in CAP.[34] These pathogens are difficult to detect via culture due to their fragility.[27] Furthermore, *Candida albicans* was the most predominant fungal pathogen detected in CAP patients by the mNGS-based approach. This observation agrees with others who show that *Candida albicans* is the most prevalent fungus in BALF samples from ventilator-associated pneumonia patients.[35] It has been reported *Pneumocystis jirovecii* cannot readily be cultured in the laboratory, which is consistent with our finding, as *Pneumocystis jirovecii* was only identified by mNGS in this study.[36] Furthermore, the most frequently detected viruses included *Human herpesvirus 4* and *Human herpesvirus 7*, which were not found to be associated with prognosis in the lower respiratory tract of patients with severe pneumonia in a previous study.[37] With its superior microbial detection rate, mNGS exhibits enormous potential in guiding antibiotic selection, monitoring the pathogen infection in CAP patients unresponsive to empirical therapy.

Pathogens are frequently found in a polymicrobial environment in vivo, suggesting that clinically significant organisms may not act alone in causing disease. Instead, they may impact pathogenesis by altering the microenvironment to facilitate colonization or by promoting the expression of virulence genes in other bacteria.[38–40] mNGS has the apparent advantages of identifying both polymicrobial infections and unknown infection sources. In this study, mNGS identified 47.62% (80/168) of patients with polymicrobial infection, which primarily multiple bacterial infections, bacterial-fungal infections, and bacterial-viral infections. Notably, 6 patients exhibited infections involving bacteria, fungi, and viruses simultaneously. *Candida* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are commonly found as

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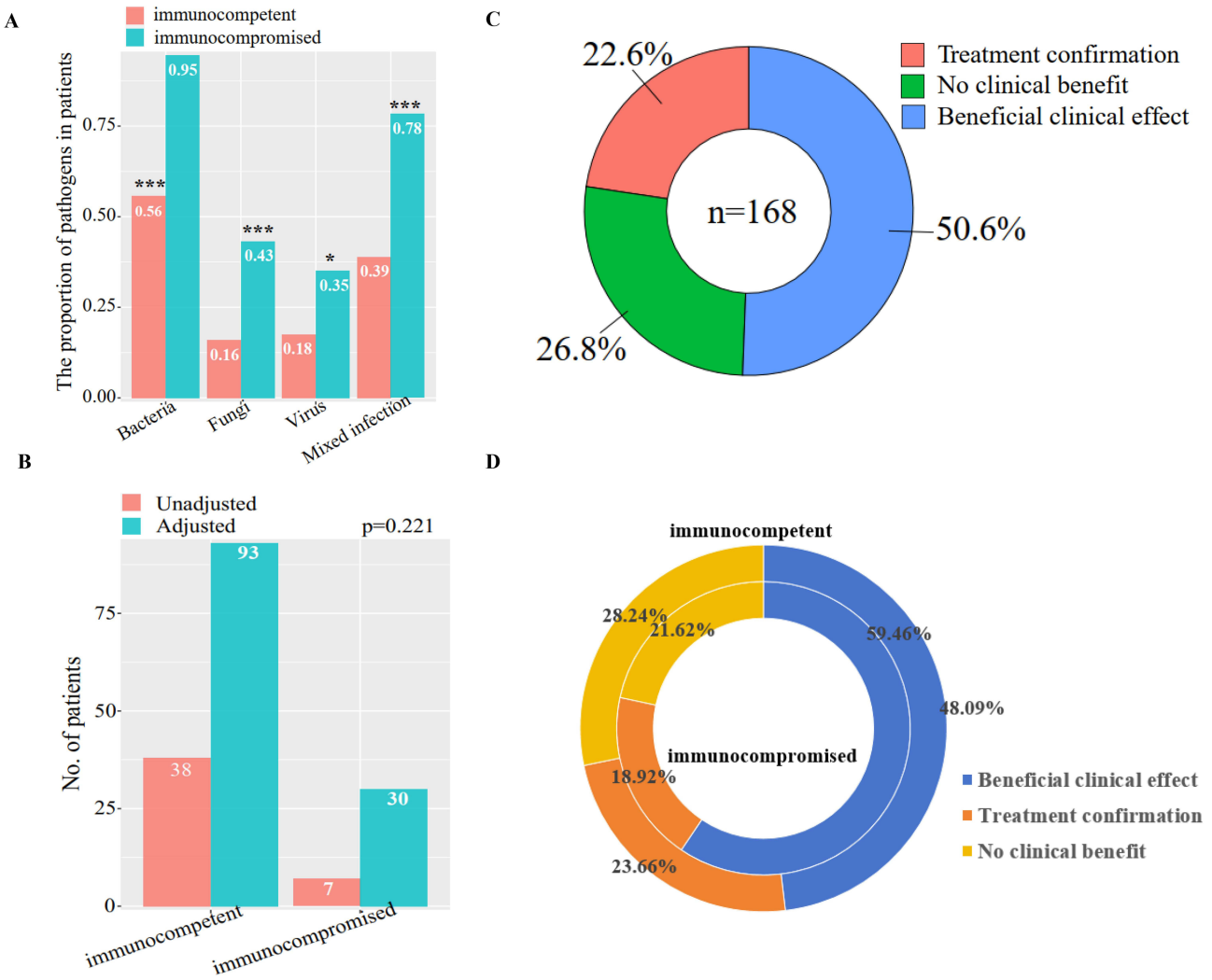

Figure 4 The comparison of microbiological pathogens between immunocompromised patients and immunocompetent patients and mNGS-guided antibiotic treatment adjustments in patients. (**A**) The positive rate difference of bacteria, fungi virus and mixed infection in immunocompromised patients compared to immunocompetent patients. *p value < 0.05; ***p value < 0.001. (**B**) The antibiotic treatment adjustment based on mNGS. (**C**) Clinical effect of mNGS on treatment in 168 patients with CAP. (**D**) Clinical effect evaluation of mNGS on treatment in immunocompromised (inner circle) and immunocompetent patients (outer circle).

commensals and colonize human mucosal surfaces. Several studies have demonstrated that the interactions of these opportunistic pathogens can enhance disease severity in various ways.[41–43]

Our study emphasizes the incidence rates of bacteria fungi, viruses and polymicrobial infections in patients with immunosuppression were significantly higher compared to those in immunocompetent patients. This finding is consistent with a previous report on the incidence rates in bacterial, fungal and viral infections, with the exception of polymicrobial infections.[44] The spectrum of pathogens in CAP differed between immunocompetent and immunocompromised patients. In immunocompromised patients, *Gemella haemolysans, Acinetobacter baumannii* and *Pneumocystis jirovecii* were the most frequently detected pathogens. *Pneumocystis jirovecii* was reported to account for 61.2% of confirmed pneumonia cases in immunocompromised patients,[12] a obviously higher proportion compared to our findings (6/37, 16.2%). This discrepancy may reflect differences in patient populations, diagnostic criteria, or regional pathogen prevalence. Additionally, mNGS showed remarked advantages for detecting *Pneumocystis jirovecii* compare to culture, as emphasized in the study by Peng et al.[12] Moreover, *Acinetobacter baumannii* infections were found to be significantly more prevalent among patients in the intensive care unit (ICU) compared to those in non-ICU patients with suspected pulmonary infection.[45] Similarly, the number of cases of *Acinetobacter baumannii* infection *were higher in* immunocompromised patients *compared to* immunocompetent patients. Additionally, immunocompromised patients have a more

Table 3 The Antibiotic Treatment Adjustment by the Diagnosis of mNGS and Clinical Impact in Immunocompromised Patients and Immunocompetent Patients

Clinical Impact	No. (%) of Samples		
	Immunocompetent (n=131)	Immunocompromised (n=37)	Total (n=168)
Adjustment			
New diagnosis	74(56.49%)	25(67.57%)	99(58.93%)
Medication changed			
Beneficial clinical effect	63(48.09%)	22(59.46%)	85(50.60%)
Discontinued	20 (15.27%)	5(13.51%)	25(14.88%)
No clinical benefit	10(7.63%)	3(8.11%)	13(7.74%)
Unadjustment			
Medication			
Continued treatment (effective outcome)	11(8.40%)	2(5.40%)	13(7.74%)
Empirical treatment (effective outcome)	24(18.32%)	5(13.51%)	29(17.26%)
False-positive result led to incorrect diagnosis	2(1.53%)	0(0%)	2(1.19%)
Incorrect antibiotic treatment	1(0.76%)	0(0%)	1(0.60%)

complex microbial etiology and are particularly susceptible to polymicrobial infections or uncommon pathogens. For example, one immunocompromised patient presented with an uncommon polymicrobial infection, including *Pneumocystis jirovecii* co-infected with *Gemella sanguinis, Torque teno virus, Mycobacterium avium* and *human herpesvirus 5*. Guided by mNGS results for pathogen detection in clinical decision-making, mNGS demonstrated a positive impact in 58.93% of cases, which was higher than the findings reported by Zhu et al[46] and Lee et al.[47] Moreover, 22 (59.46%) of 37 immunocompromised patients and 63 (48.09%) of 131 immunocompetent patients obtained beneficial clinical effect (initiation of targeted treatment and treatment de-escalation). However, there was no significant difference in antibiotic treatment adjustment between both groups in our study ($p = 0.221$). These findings are consistent with the results of a previous study about severe CAP in immunocompromised adults conducted by Sun et al.[48] Another study reports 48.6% of critically iII patients with immunocompromission experienced beneficial clinical effect based on mNGS results.[44] As such, mNGS has great potential for targeted therapy and improved the management of empirical antimicrobial overuse in immunocompromised patients with CAP.

There were some limitations in our study. First, it was a single–center study with a small sample size in both the immunocompetent patients and immunocompromised patients, and the results may be biased compared to multicenter studies with large sample sizes. Second, the cost of mNGS is high, with a single detection compared to culture method, while it may help shorten the time for a definitive diagnosis and increase the positive rate of pathogens. Third, the patients in this study received empirical antimicrobial treatment prior to mNGS and culture, which may have led to changes in the bacteria, fungi, viruses and lower clinical impact. Furthermore, the exclusion of microorganisms based on an in-house background database could further contribute to bias. Finally, we only performed DNA sequencing in our study and did not include RNA mNGS tests, which may have led to an underestimation of the performance of mNGS in pathogen identification. Therefore, it is necessary that future studies conduct multicenter explorations with large samples to address these limitations.

Conclusion

Collectively, mNGS significantly outperformed culture in improving the diagnostic rate of infections, particularly for pathogens such as *Streptococcus pneumoniae, Candida albicans* and *Human herpesvirus*. BALF mNGS demonstrated its notable advantages in detecting pathogenic microbes missed by sputum culture, which is of great significance for guiding the usages of antibiotics.

Data Sharing Statement

All datasets presented in the current study are available in the NCBI repository, http://www.ncbi.nlm.nih.gov/bioproject/1131080; BioProject ID: PRJNA1131080.

Ethics Approval

The research involving human participants underwent review and approval by the Ethics Committee of the Affiliated Hospital of Chengde Medical College. The ethical number is No.CyFyLL2020262. Informed consent was obtained from all individuals included in the study.

Consent for Publication

All authors gave consent for publication.

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Disclosure

Pei Peng and Yaoyao Wang are affiliated to Shanghai Biotecan Pharmaceuticals Co., Ltd. The authors confirm that the study was conducted without any other commercial or financial relationships that could be perceived as a potential conflict of interest.

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