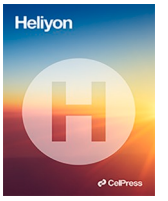
Heliyon 10 (2024) e35287

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Clinical impact of metagenomic next-generation sequencing of bronchoalveolar lavage fluids for the diagnosis of pulmonary infections in respiratory intensive care unit

Heng Zhang [a,1], Ming Lu [b,1], Chaomin Guo [c], Lifeng Wang [c], Kun Ye [c], Qiang Zhao [c], Jiyong Yang [c], Liuyang Yang [c,**], Tanshi Li [a,*]

[a] Department of Emergency, The First Medical Center, Chinese PLA General Hospital, Medical School of Chinese PLA, Beijing, 100853, China
[b] Laboratory Medicine Department, Tangshan Nanhu Hospital, Hebei, 063000, China
[c] Laboratory Medicine Department, The First Medical Center, Chinese PLA General Hospital, Beijing, 100853, China

ARTICLE INFO

Keywords:
Bronchoalveolar lavage fluid
Metagenomic next-generation sequencing
Pulmonary infections

ABSTRACT

Background: The real-world clinical impact of mNGS on BALF in the respiratory intensive care unit (RICU) is not yet fully understood.
Methods: We investigated the clinical impact of mNGS on BALF samples obtained from 92 patients admitted to the RICU over a 2-year period. We utilized both mNGS and culture methods to evaluate the effectiveness of mNGS in diagnosing pulmonary infections. The clinical impact of mNGS were evaluated by the clinician committees.
Results: Among the 92 diagnosed patients, 78 cases (84.7 %) were determined to have infectious diseases caused by pathogenic microorganisms, and the bacterial infections constituted the most prevalent diagnostic category. For mixed infection, the most common type was the *Pneumocystis jironecii* and cytomegalovirus co-infection. The mNGS results had a positive impact on the clinical management of 43 cases (46.7 %). Moreover, 19 cases (44.2 %) of positive clinical impacts were solely based on new diagnoses made possible by mNGS results. These new diagnoses were particularly helpful for identifying rare pathogens, which could not be detected by conventional diagnostic methods.
Conclusions: The BALF mNGS has a positive real-world impact in RICU. Clinician committee play a critical role in ensuring the appropriate use of mNGS.

1. Introduction

Metagenomic next-generation sequencing (mNGS) is a rapid microbiological method for diagnosing infectious diseases, providing a comprehensive array of etiological insights encompassing species, strains, antibiotic resistance, and pathogenic characteristics [1,2]. Its successful integration has been observed across various clinical domains, including respiratory, hematology, neurosurgery, orthopedics, and hepatobiliary surgery [3]. Notably, the respiratory intensive care unit (RICU) serves as a pivotal setting for patients with

* Corresponding author.
** Corresponding author.
E-mail addresses: yang3608@foxmail.com (L. Yang), lts301@163.com (T. Li).
[1] Heng Zhang and Ming Lu are co-first authors and contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e35287
Received 4 December 2023; Received in revised form 23 July 2024; Accepted 25 July 2024
Available online 26 July 2024

moderate to severe respiratory failure, offering non-invasive respiratory support to potentially circumvent ICU admission [4]. Bronchoalveolar lavage fluid (BALF) remains the premier specimen for pinpointing pathogens underlying lung infections. Nevertheless, despite extensive microbiological scrutiny, the causative agent remains elusive in over 60 % of cases [5], primarily due to the lack of diagnostic testing for rare pathogens and the constraints of culture-based testing methods.

Since its inaugural clinical application in 2014, mNGS has shown promise in diagnosing infectious diseases, owing to its ability to identify multiple pathogens in a single assay [6]. When applied to clinical practice, studies have reported a sensitivity and specificity of 50.7 and 85.7 %, respectively, for diagnosing infectious diseases through mNGS [7]. Moreover, mNGS has demonstrated superiority over culture-dependent methods in various aspects, particularly in detecting *Mycobacterium tuberculosis* (MTB), viruses, anaerobes, and fungi [8]. However, the broader clinical ramifications of mNGS testing on routine patient care remain elusive. Published studies detailing the efficacy of mNGS in individuals experiencing pulmonary inflammation have predominantly been limited to isolated instances or small-scale retrospective case series [9–14]. Therefore, it remains uncertain whether the diagnostic prowess and efficiency of mNGS tests for lung inflammation can be extrapolated more comprehensively and judiciously in clinical practice.

The emergence of clinical metagenomic cell-free DNA sequencing has generated considerable interest among healthcare professionals in various fields, owing to its non-invasive characteristics and ability to facilitate quicker actionable diagnoses than traditional microbiological techniques. However, the true clinical impact of mNGS testing in routine patient care remains largely uncharted. In our study, we conducted a two-year retrospective analysis of hospitalized patients in the respiratory care unit afflicted with single pulmonary infections, respiratory failure, and bronchiectasis.

2. Methods

2.1. Study design and patient cohort

Between October 1, 2019, and October 31, 2021, a total of 92 patients were screened for review and enrollment in this study after being admitted to the Respiratory Intensive Care Unit (RICU) at the First Medical Center of the People's Liberation Army General Hospital in Beijing, China (Table 1). Patients were enrolled in this retrospective study to validate the mNGS assay if they had at least

Table 1
Clinical characteristics of 92 patients included.

Characteristics, n (%)	92 patients
Age	
Mean (year)	62
Distribution, n (%)	
<20	2
21-40	13
41-60	19
61-70	26
71-80	14
81-90	10
>90	8
Gender, n (%)	
Male	58 (63.0)
female	34 (37.0)
Syndrome, n (%)	
Fever	72 (78.3)
Radiographic abnormality	20 (21.7)
Clinical diagnosis, n (%)	
Infectious disease	80 (87.0)
Non-infectious diseases	12 (13.0)
Immunity, n (%)	
Normal	70 (76.1)
Immunocompromised	22 (23.9)
Comorbidities, n (%)	
Hypertension	27 (29.3)
Cardiovascular disease	23 (25)
Diabetes	21 (22.8)
Tumor surgery	18 (19.6)
Kidney disease	15 (16.3)
Anemia	17 (18.5)
Diseases of the blood system	13 (14.1)
Organ dysfunction	13 (14.1)
Autoimmune disease	12 (13)
After graft transplantation	5 (5.4)
ICU outcome, n (%)	
Improved	41 (44.5)
Death	18 (19.5)
Indeterminate	33 (36.0)

one of the following: 1) fever of undetermined origin [15], 2) ineffective antibiotic treatment, 3) radiographic abnormality, or 4) body temperature exceeding 38.3 °C with pathogenic microorganisms undetected by routine testing methods including culture, PCR targeting to CMV, EBV, 2019-nCoV, influenza A virus, and influenza B virus, and imaging.

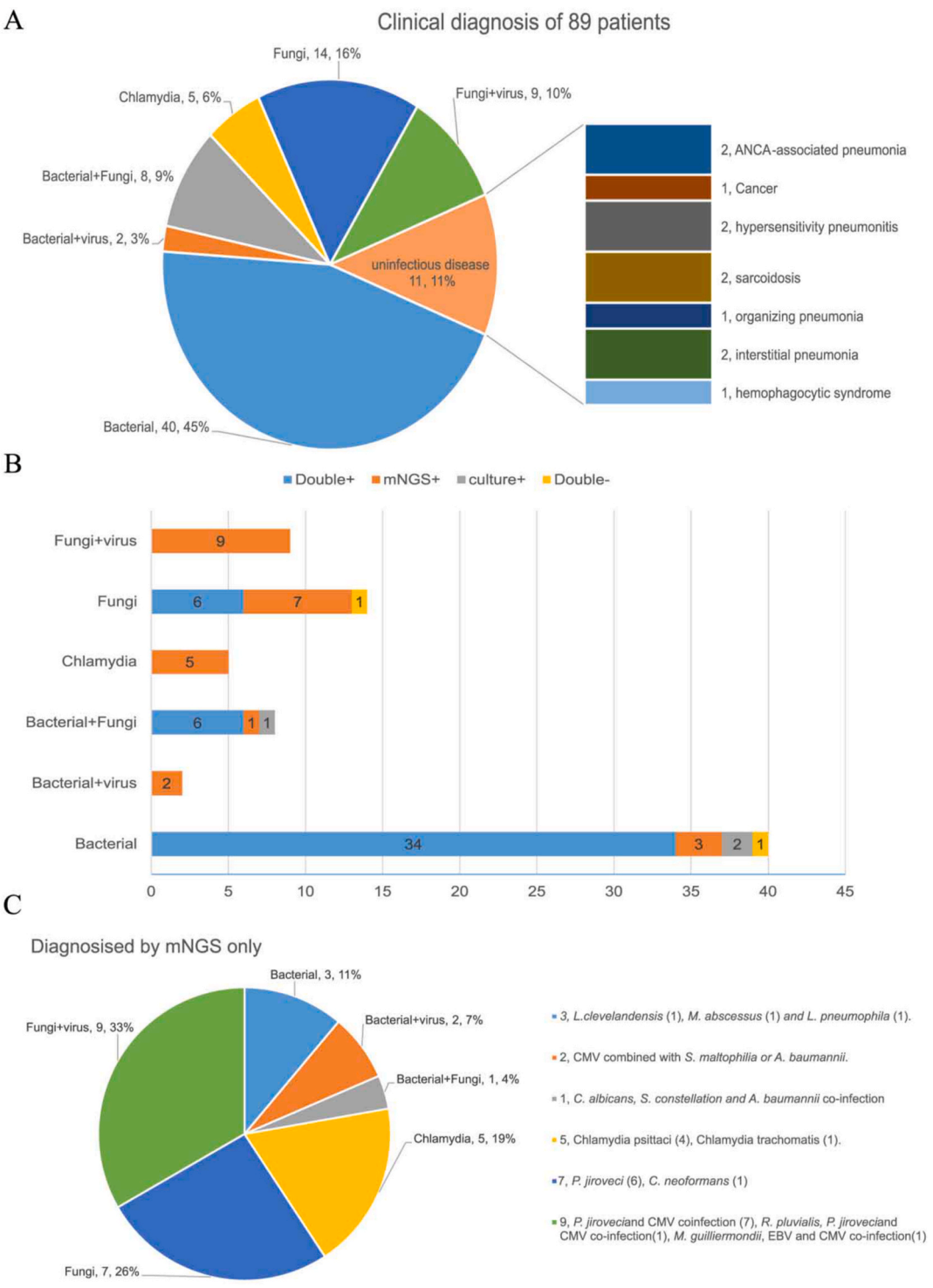


Fig. 1. Clinical diagnosis of 89 patients in RICU. (A) Clinical diagnosis of the 89 patients in RICU by clinician committees. (B) The overlap of positivity between mNGS technique and culture methods for different infectious pathogens profiles. (C) Percentage of patients with mixed infections for various pathogens.

2.2. mNGS of bronchoalveolar lavage fluid (BALF)

We performed metagenomic DNA sequencing (metaDNA-seq) and/or metatranscriptomic sequencing (metaRNA-seq) on clinical BALF samples, adhering to established protocols [16]. For the metaDNA-seq process, DNA was extracted utilizing the QIAamp® UCP Pathogen DNA Kit (Qiagen) according to the manufacturer's guidelines [17]. In the case of metaRNA-seq, total RNA was obtained using the QIAamp® Viral RNA Kit (Qiagen). Following the removal of ribosomal RNA with the Ribo-Zero rRNA Removal Kit (Illumina), cDNA synthesis was carried out using reverse transcriptase and deoxynucleotide triphosphates (Thermo Fisher). Libraries for both DNA and cDNA were prepared with the Nextera XT DNA Library Prep Kit (Illumina) [18], pooled together, and subsequently sequenced on an Illumina NextseqCN500 platform for 75 cycles of single-end sequencing, aiming to yield approximately 20 million reads per library. Concurrently, peripheral blood mononuclear cells (PBMCs) from healthy donors, at a concentration of 10^5 cells/mL, along with sterile deionized water, were processed as negative and non-template controls (NTC), respectively [8]. Quality control measures involved the removal of low-quality reads, adapter contamination, duplicate reads, and any sequences shorter than 50 bp, accomplished through Trimmomatic [19]. Reads exhibiting low complexity were filtered out using Kcomplexity with its default settings. Human sequences were identified and excluded by aligning reads to the human reference genome (hg38) via Burrows-Wheeler Aligner software [20]. To construct a reference database, genomes for human (hg38) and various organisms—including 26,207 bacterial, 308 archaeal, 54 fungal, 9021 viral, 178 invertebrate, and 39 protozoan species—were sourced from the National Center for Biotechnology Information (https://ftp.ncbi.nlm.nih.gov/genomes/) and processed using Kraken2 v2.0.8beta. Taxonomic classification was performed using Kraken2 [21], and species-level quantification of reads was executed with Bracken using default parameters [22]. For pathogens identified in the negative control, a species or genus was considered positively detected if the Revolutions Per Minute (RPM) ratio was $\geq$10, defined as the RPM of the sample divided by the RPM of the NTC [18]. For pathogens absent from the negative control, the RPM threshold was set at $\geq$0.05. Additionally, penalties of 5 % for species and 10 % for genus were applied [23].

2.3. Clinical diagnoses by clinician committees

The clinician committees, comprising respiratory medicine clinicians, clinical laboratory physicians, and radiologists, were responsible for all infection diagnoses. The diagnoses were based on clinical symptoms, lung imaging findings, routine testing results (culture, PCR, lung imaging, etc.), and mNGS results.

2.4. Clinical impact evaluation

The clinician committee categorized the clinical impact of the identified microorganisms as previously reported [24]. All classifications were determined by the clinician committees and documented in the patient's medical records. The classification of clinical impact included "positive," "none," or "indeterminate." A "positive" clinical impact indicated that the identified microorganisms were deemed causative agents of the respiratory infection, prompting the initiation of clinical treatment. A "none" clinical impact meant that the identified microorganisms were unlikely contributors to the patient's respiratory infection. An "indeterminate" clinical impact suggested that the identified microorganisms might have played a role in the respiratory infection, but further testing and clinical evaluation were necessary to confirm the causality.

2.5. Statistical analysis

No formal calculations for sample size were conducted because of the review's descriptive nature.

3. Results

3.1. Clinical diagnoses and pathogen detection

Out of the 92 patients, 89 were diagnosed by the clinician committee, with the remaining three discharged before mNGS results were available. Among the diagnosed patients, 84.7 % (n = 78) were determined to have infectious diseases caused by pathogenic microorganisms, whereas 12.0 % (n = 11) were ruled out of infectious diseases (Fig. 1A). Bacterial infections (51.3 %) constituted the most prevalent diagnostic category, followed by fungi (17.9 %) and chlamydia (6.4 %). Most infected patients (75.6 %) were diagnosed with a single microorganism infection, while 24.4 % had mixed infections of fungi-viruses or bacteria-fungi (Table S1). Viruses, chlamydia, and certain fungi (e.g., *Pneumocystis jironecii* and *Cryptococcus neoformans*) were mainly detected in BALF using mNGS testing (Table S1; Fig. 1B). The most common type of mixed infection detected by mNGS was viruses-fungi co-infection (n = 9), such as *P. jironecii* and cytomegalovirus (CMV) (Fig. 1B).

The most frequently detected pathogenic bacteria were *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Table S1). Eighty-five percent of bacterial infections were detected by both mNGS and conventional culture methods (Fig. 1B). *P. jironecii* (n = 6), *Chlamydia psitsiti* (n = 4), *C. neoformans*, *Lawsonella clevelandensis*, *Mycobacterium abscessus*, and *Legionella pneumophila* were solely detected by mNGS and were remained undetectable by culture methods. While two cases were diagnosed with *S. aureus* or *A. baumannii* using culture methods, mNGS only reported *Staphylococcus epidermidis*. One case of *Mycobacterium tuberculosis* infection was undetected by either method but was diagnosed by clinician committees based on typical clinical symptoms, imaging

diagnosis, and effective treatment measures (Table S1). Despite the detection of *P. jironecii* in the BALF of a 34-year-old male using mNGS, the clinician committees did not support the diagnosis of *P. jironecii* pneumonia based on imaging data. Another case was diagnosed with *C. neoformans* pneumonia based on clinical symptoms, imaging diagnosis, and a positive result for the *C. neoformans* antigen, which was not reported by mNGS. Additionally, one patient diagnosed with *Candida auris* pneumonia using both methods unfortunately passed away despite aggressive measures.

3.2. Non-infectious diseases

The 11 cases of non-infectious diseases are listed in Table S2. Among them, only two mNGS results did not report any pathogenic microorganisms. The remaining nine microorganisms detected by mNGS (81.8 %) included *Haemophilus influenzae*, *A. baumannii*, *Enterococcus faecalis*, *Enterococcus faecium* and *K. pneumoniae*. However, these microorganisms were considered as colonization rather than pathogenic.

3.3. Diagnosis of infectious diseases solely by mNGS

Twenty-seven cases were diagnosed solely by mNGS, mainly consisting of *P. jirovecii*, *C. psitsiti*, and CMV (Fig. 1C). Additionally, rare pathogens such as *L. clevelandensis*, *M. abscessus*, *Streptococcus constellatus*, *Chlamydia trachomatis*, *Rhizomucor pluvialis,* and *Meyerozyma guilliermondii* were solely detected by mNGS, eluding detection by conventional culture methods.

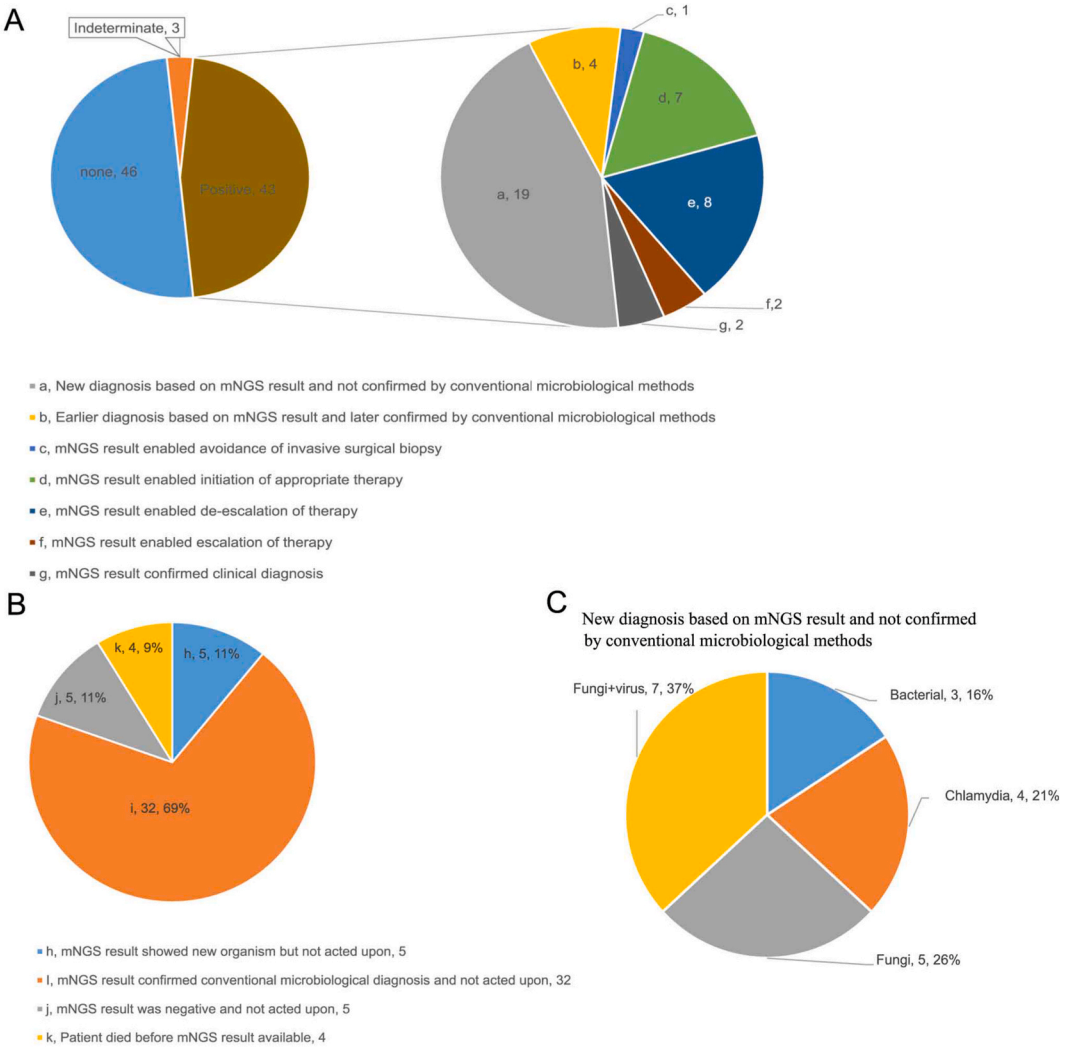


Fig. 2. Clinical impact of BALF mNGS in RICU. The percentage of patients with the positive clinical impact (A) and nonclinical impact (B). The profiles in the 19 new diagnoses solely detected by mNGS in the positive clinical impact (C).

3.4. Clinical impact of mNGS for BALF

Among the total 92 patients, mNGS results led to a "positive" clinical impact in 43 patients (46.7 %), whereas "none" and "indeterminate" clinical impacts were observed in 46 patients (50.0 %) and three patients (3.3 %), respectively (Fig. 2A). "Positive" clinical impacts were categorized as new diagnoses solely based on mNGS results (n = 19), earlier diagnoses than conventional methods (n = 4), avoidance of invasive surgical biopsy (n = 1), initiation of appropriate therapy (n = 7), de-escalation of therapy (n = 8), escalation of therapy (n = 2), and confirmed clinical diagnosis (n = 2; Fig. 2A). A 63-year-old female patient with multiple microorganism co-infections (*M. guilliermondii*, EBV, and CMV) was successfully cured with fluconazole and thymopentin therapy based on the mNGS results. Needle biopsy for a 65-year-old patient was avoided due to the detection of *C. psitsiti*, resulting in the clinical impact of avoiding invasive surgical biopsy (Table S2).

The 19 new diagnoses solely based on mNGS included fungi (n = 5), chlamydia (n = 4), bacteria (n = 3) and fungi-virus co-infection (n = 7, Fig. 2C). The most frequently detected microorganisms by mNGS were *P. jerovecii* (n = 5), *C. psitsiti* (n = 3), *M. abscessus* (n = 1), *L. pneumoniae* (n = 1), CMV (n = 1), and *L. clevelandensis* (n = 1). Moreover, all seven fungi-virus co-infection cases were *P. jerovecii* combined with CMV. These rare pathogenic microorganisms were only detected by mNGS rather than routine testing methods.

Out of the 46 cases with no clinical impact, 32 patients (69.7 %) had confirmed conventional microbiological diagnoses that did not require further action, five patients had a new organism detected but not acted upon, five patients had negative mNGS results that were not acted upon, and four patients died before mNGS results were available (Fig. 2B).

4. Discussion

Given the potential of mNGS to detect a wide variety of pathogens, it has garnered great interest among medical subspecialties [25,26]. However, previous reports have indicated limited clinical impact of mNGS in diagnosing blood and cerebrospinal fluid infections, with positive clinical impact reported at 5.4 % and 7.3 %, respectively [24,27]. Detecting pathogens in BALF is crucial for respiratory diseases, and mNGS has been utilized in numerous studies [28,29]. Nevertheless, the clinical utility of mNGS testing as a diagnostic tool in the RICU remains poorly understood. This study systematically and retrospectively evaluated the clinical usefulness of BALF mNGS for diagnosing pulmonary infections in the RICU.

Accurate clinical diagnosis is critical for determining clinical decisions. In this study, clinician committees ensured the accuracy of clinical infection diagnosis. Despite the promising clinical application prospects of mNGS [30], result interpretation is challenging, particularly in distinguishing between colonization and infection, especially in patients with multiple comorbidities and suspected intestinal diseases [10]. Given the serious complications observed in many RICU patients in this study (Table 1), clinician committees played a crucial role in ensuring accurate clinical infection diagnoses. Many institutions/clinics have established clinician committees, multidisciplinary teams, or clinical microbial sequencing boards to aid in clinical diagnosis [27,31,32], emphasizing the importance of obtaining results based on clinical symptoms, lung imaging findings, routine testing methods, and mNGS.

The diagnosis in this study was determined by the clinician committees (Fig. 1). Several studies have demonstrated consistency between mNGS and culture tests in detecting bacteria and fungi [33]. While mNGS incurs significantly higher costs compared to traditional culture methods or conventional PCR, its ability to detect a wide range of pathogens may justify its use. In this study, bacterial (45 %) and fungal (16 %) infections accounted for a substantial proportion of pathogen infections in the RICU, with 85.0 % of bacterial and 42.9 % of fungal infections detected by both mNGS and culture tests (Fig. 1). However, a small portion of bacterial and nearly half of fungi infections were undetected by culture methods. Prior antibiotic treatments before BALF sample collection may lead to false-negative culture results [34,35], whereas mNGS is less likely to be affected by prior antibiotic usage [7]. Additionally, fungal infections solely detected by mNGS were predominantly induced by *P. jironecii*, a pathogen often missed by culture methods [36]. Although real-time PCR has shown high specificity in detecting *P. jironecii* in blood and BALF samples, its routine clinical usage is challenging [37]. Researchers have demonstrated that mNGS could improve virus detection in central nervous system diseases or respiratory-related diseases [38,39]. In this study, we observed seven cases of co-infection of *P. jironecii* and CMV, with four cases successfully treated due to timely clinical intervention. Additionally, clinician committees diagnosed several rare or opportunistic pathogens solely based on the mNGS results (Fig. 1C; Table S1). Therefore, mNGS could be considered a reliable detection method for BALF, particularly for identifying rare pathogenic microorganisms such as chlamydia and certain fungi.

While research on BALF mNGS has proliferated in recent years [33,39,40], attention has primarily focused on sensitivity and specificity differences between mNGS and routine testing methods, with less emphasis on clinical impact. In our retrospective cohort study, we observed that the real-world clinical impact of mNGS, as it is currently implemented in clinical practice, positively influenced nearly 50.0 % of the patients (Fig. 2A). This finding is inconsistent with the limited clinical effectiveness of mNGS in multicenter retrospective cohort studies, whereas 92.7 % of plasma samples and 96.1 % of CSF samples showed limited real-world clinical impact [24,27]. These studies further demonstrated that mNGS assays conducted on CSF and plasma exhibit lower sensitivity when compared to traditional methods. Consequently, mNGS should not be utilized as standalone tests or for ruling out conditions.

Several factors may explain the observed discrepancy. Firstly, differences in sample types may drive this disparity, as BALF is a relatively easy-to-obtain clinical sample with higher clinical diagnostic value compared to others [40]. The high host background of the blood and CSF samples may increase the risk of false-negative results, warranting cautious interpretation by clinician committees [27]. Secondly, patient selection practices may have played a role in the significant clinical impact of BALF mNGS observed in this research. All patients included for mNGS testing were selected solely based on clinician committee interpretation, whereas in other studies, patient selection from medical records was unclear, potentially leading to invalid mNGS detection. Thirdly, clinical decision-making by clinician committees likely enhanced the clinical impact of mNGS. mNGS's ability to detect multiple potential

pathogenic microorganisms in a single assay, independent of a prior selection of target pathogens, complicates determining whether a microbe is dead or alive [41]. Clinician committees in this study determined the clinical significance of mNGS results, influencing patient management.

Nevertheless, approximately half of the samples yielded none or indeterminate clinical impact, where BALF mNGS results were either not considered by treating clinicians or had no impact on conventional testing. BALF samples from the respiratory tract often contain oral flora and colonizing bacteria, resulting in a wider range of microbial species detected by mNGS. mNGS identifies cell-free DNA in a sample, enabling the detection of DNA from previously present but inactive non-viable bacteria [42]. Consequently, mNGS results may detect new organisms or confirm conventional microbiological diagnoses but not result in further action.

Although mNGS or routine tests detected microorganisms in 11 patients without infectious diseases, clinician committees should also consider the possibility of non-infectious conditions, such as cancer or ANCA-associated pneumonia. Diagnosis based solely on mNGS results without considering the primary disease may lead to inappropriate or unnecessary treatments, resulting in patients suffering and financial burden [24].

However, there are still some limitations to consider. Firstly, although clinician committees determined the clinical impact, some cases were medically complex, making interpretation challenging. This could introduce bias in the assessment of clinical effectiveness. A thorough and impartial assessment of factors like antibiotic usage, duration of stay, and the use of laboratory tests is essential. Secondly, the criteria for selecting samples for mNGS were not uniformly applied. This variability in patient selection may affect the assessment of clinical impact due to differences in pre-test probabilities.

In summary, BALF mNGS serves as a promising diagnostic tool due to its ability to deliver timely and actionable diagnoses. Our study demonstrated a positive real-world impact of mNGS in RICU, with almost half of the cases showing positive clinical impact. The diagnostic accuracy of clinician committees is crucial for maximizing the clinical impact of mNGS, as it can reduce unnecessary testing and improve report interpretation. Further research is necessary to enhance our understanding of which patient groups are likely to gain the most from mNGS alongside traditional microbiological approaches. Additionally, clinician committees should play an active role in diagnostic management to ensure optimal utilization of mNGS.

Funding

This research received support from the National Key Research and Development Program of China (No. 2019YFF0302300).

Ethics statement

This study was approved by the institutional review boards at First Medical Center of the People's Liberation Army General Hospital (Ethical approval number: S2024-079-01).

Consent for publication

The patients consented to the publication of their data and images in this study.

Data availability statement

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

CRediT authorship contribution statement

Heng Zhang: Writing – original draft, Data curation. **Ming Lu:** Writing – original draft, Methodology, Data curation. **Chaomin Guo:** Writing – review & editing. **Lifeng Wang:** Methodology, Data curation. **Kun Ye:** Visualization, Data curation. **Qiang Zhao:** Data curation. **Jiyong Yang:** Data curation. **Tanshi Li:** Writing – review & editing, Writing – original draft, Funding acquisition. **Liuyang Yang:** Writing – review & editing, Writing – original draft, Project administration, Data curation.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of the manuscript entitled.

Acknowledgements

We thank all participants for their time and effort.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35287.

References

[1] I. Moreno, et al., The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology, Am. J. Obstet. Gynecol. 218 (6) (2018) 602.e1–602.e16.
[2] C. Langelier, et al., Metagenomic sequencing detects respiratory pathogens in hematopoietic cellular transplant patients, Am. J. Respir. Crit. Care Med. 197 (4) (2018) 524–528.
[3] D. Han, et al., mNGS in clinical microbiology laboratories: on the road to maturity, Crit. Rev. Microbiol. 45 (5–6) (2019) 668–685.
[4] V. Di Lecce, et al., Baseline characteristics and outcomes of COVID-19 patients admitted to a respiratory intensive care unit (RICU) in southern Italy, Multidiscip Respir Med 15 (1) (2020) 704.
[5] R. Schlaberg, et al., Validation of metagenomic next-generation sequencing tests for universal pathogen detection, Arch. Pathol. Lab Med. 141 (6) (2017) 776–786.
[6] M.R. Wilson, et al., Actionable diagnosis of neuroleptospirosis by next-generation sequencing, N. Engl. J. Med. 370 (25) (2014) 2408–2417.
[7] Q. Miao, et al., Microbiological diagnostic performance of metagenomic next-generation sequencing when applied to clinical practice, Clin. Infect. Dis. 67 (suppl_2) (2018) S231–s240.
[8] H. Li, et al., Detection of pulmonary infectious pathogens from lung biopsy tissues by metagenomic next-generation sequencing, Front. Cell. Infect. Microbiol. 8 (2018) 205.
[9] F. Xie, et al., Clinical metagenomics assessments improve diagnosis and outcomes in community-acquired pneumonia, BMC Infect. Dis. 21 (1) (2021) 352.
[10] C.Y. Chiu, S.A. Miller, Clinical metagenomics, Nat. Rev. Genet. 20 (6) (2019) 341–355.
[11] X. Jin, et al., Improving suspected pulmonary infection diagnosis by bronchoalveolar lavage fluid metagenomic next-generation sequencing: a multicenter retrospective study, Microbiol. Spectr. 10 (4) (2022) e0247321.
[12] J. Wang, Y. Han, J. Feng, Metagenomic next-generation sequencing for mixed pulmonary infection diagnosis, BMC Pulm. Med. 19 (1) (2019) 252.
[13] Y.Y. Qian, et al., Improving pulmonary infection diagnosis with metagenomic next generation sequencing, Front. Cell. Infect. Microbiol. 10 (2020) 567615.
[14] G. Xie, et al., Exploring the clinical utility of metagenomic next-generation sequencing in the diagnosis of pulmonary infection, Infect. Dis. Ther. 10 (3) (2021) 1419–1435.
[15] G.A. Jacoby, M.N. Swartz, Fever of undetermined origin, N. Engl. J. Med. 289 (26) (1973) 1407–1410.
[16] Y. Tao, et al., Diagnostic performance of metagenomic next-generation sequencing in pediatric patients: a retrospective study in a large children's medical center, Clin. Chem. 68 (8) (2022) 1031–1041.
[17] Y. Amar, et al., Pre-digest of unprotected DNA by Benzonase improves the representation of living skin bacteria and efficiently depletes host DNA, Microbiome 9 (1) (2021) 123.
[18] S. Miller, et al., Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid, Genome Res. 29 (5) (2019) 831–842.
[19] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (15) (2014) 2114–2120.
[20] H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform, Bioinformatics 25 (14) (2009) 1754–1760.
[21] D.E. Wood, S.L. Salzberg, Kraken: ultrafast metagenomic sequence classification using exact alignments, Genome Biol. 15 (3) (2014) R46.
[22] R. Kobus, et al., A big data approach to metagenomics for all-food-sequencing, BMC Bioinf. 21 (1) (2020) 102.
[23] W. Gu, et al., Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids, Nat. Med. 27 (1) (2021) 115–124.
[24] C.A. Hogan, et al., Clinical impact of metagenomic next-generation sequencing of plasma cell-free DNA for the diagnosis of infectious diseases: a multicenter retrospective cohort study, Clin. Infect. Dis. 72 (2) (2021) 239–245.
[25] W. Gu, S. Miller, C.Y. Chiu, Clinical metagenomic next-generation sequencing for pathogen detection, Annu. Rev. Pathol. 14 (2019) 319–338.
[26] R.H. Deurenberg, et al., Application of next generation sequencing in clinical microbiology and infection prevention, J. Biotechnol. 243 (2017) 16–24.
[27] M. Wilson, et al., Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis, N. Engl. J. Med. 380 (24) (2019) 2327–2340.
[28] X. Chen, et al., Blood and bronchoalveolar lavage fluid metagenomic next-generation sequencing in pneumonia, Can. J. Infect Dis. Med. Microbiol. 2020 (2020) 6839103.
[29] X. Fang, et al., Diagnostic value of metagenomic next-generation sequencing for the detection of pathogens in bronchoalveolar lavage fluid in ventilator-associated pneumonia patients, Front. Microbiol. 11 (2020) 599756.
[30] T.A. Blauwkamp, et al., Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease, Nat Microbiol 4 (4) (2019) 663–674.
[31] B. Pillay, et al., The impact of multidisciplinary team meetings on patient assessment, management and outcomes in oncology settings: a systematic review of the literature, Cancer Treat Rev. 42 (2016) 56–72.
[32] K.J. Myall, et al., Persistent post-COVID-19 interstitial lung disease. An observational study of corticosteroid treatment, Ann Am Thorac Soc 18 (5) (2021) 799–806.
[33] Y. Chen, et al., Application of metagenomic next-generation sequencing in the diagnosis of pulmonary infectious pathogens from bronchoalveolar lavage samples, Front. Cell. Infect. Microbiol. 11 (2021) 541092.
[34] A.M. Harris, et al., Influence of antibiotics on the detection of bacteria by culture-based and culture-independent diagnostic tests in patients hospitalized with community-acquired pneumonia, Open Forum Infect. Dis. 4 (1) (2017) ofx014.
[35] J. Rhodes, et al., Antibiotic use in Thailand: quantifying impact on blood culture yield and estimates of pneumococcal bacteremia incidence, Am. J. Trop. Med. Hyg. 83 (2) (2010) 301–306.
[36] P. Zak, et al., BAL fluid analysis in the identification of infectious agents in patients with hematological malignancies and pulmonary infiltrates, Folia Microbiol. (Prague, Czech Repub.) 65 (1) (2020) 109–120.
[37] M.J. Alvarez-Martínez, et al., Sensitivity and specificity of nested and real-time PCR for the detection of Pneumocystis jiroveci in clinical specimens, Diagn. Microbiol. Infect. Dis. 56 (2) (2006) 153–160.
[38] A. Piantadosi, et al., Enhanced virus detection and metagenomic sequencing in patients with meningitis and encephalitis, mBio 12 (4) (2021) e0114321.
[39] C.L. Shi, et al., Clinical metagenomic sequencing for diagnosis of pulmonary tuberculosis, J. Infect. 81 (4) (2020) 567–574.
[40] Y. Li, et al., Application of metagenomic next-generation sequencing for bronchoalveolar lavage diagnostics in critically ill patients, Eur. J. Clin. Microbiol. Infect. Dis. 39 (2) (2020) 369–374.
[41] J.B. Emerson, et al., Schrödinger's microbes: tools for distinguishing the living from the dead in microbial ecosystems, Microbiome 5 (1) (2017) 86.
[42] J.W. Ai, et al., Dynamic and direct pathogen load surveillance to monitor disease progression and therapeutic efficacy in central nervous system infection using a novel semi-quantitive sequencing platform, J. Infect. 76 (3) (2018) 307–310.