Supplementary Results

Supplementary Table 1: List of included studies in the systematic review.

PMID	Authors	Journal	Year	Title	
35943274	Jin X et al.	Microbiol Spectr	2022	Improving Suspected Pulmonary Infection Diagnosis by Bronchoalveolar Lavage Fluid Metagenomic Next-Generation Sequencing: a Multicenter Retrospective Study	[1]
33858378	Xie F et al.	BMC Infect Dis	2021	Clinical metagenomics assessments improve diagnosis and outcomes in community-acquired pneumonia	[2]
36710980	Liu Y et al.	Front Cell Infect Microbiol	2023	Metagenomics next-generation sequencing provides insights into the causative pathogens from critically ill patients with pneumonia and improves treatment strategies	[3]
34784976	Charalampous T et al.	Genome Med	2021	Evaluating the potential for respiratory metagenomics to improve treatment of secondary infection and detection of nosocomial transmission on expanded COVID-19 intensive care units	[4]
32821543	Zhang P et al.	PeerJ	2020	Metagenomic next-generation sequencing for the clinical diagnosis and prognosis of acute respiratory distress syndrome caused by severe pneumonia: a retrospective study	[5]
35071027	Yang F et al.	Front Cell Infect Microbiol	2022	Clinical Symptoms and Outcomes of Severe Pneumonia Caused by Chlamydia psittaci in Southwest China	[6]
35816485	Li X et al.	PLoS One	2022	Clinical, radiological and pathological characteristics of moderate to fulminant psittacosis pneumonia	[7]
37580698	Tang X et al.	BMC Infect Dis	2023	Psittacosis caused severe community-acquired pneumonia accompanied by acute hypoxic respiratory failure: a multicenter retrospective cohort study from China	[8]
34603275	Zhan Y et al.	Front Microbiol	2021	Clinical Evaluation of a Metagenomics-Based Assay for Pneumonia Management	[9]
36409152	Chen L et al.	Microbiol Spectr	2022	Metagenomic Next-Generation Sequencing for the Diagnosis of Neonatal Infectious Diseases	[10]
35842216	Yin Q et al.	Int J Infect Dis	2022	Atypical pneumonia caused by Chlamydia psittaci during the COVID-19 pandemic	[11]
38145053	Hao J et al.	Front Cell Infect Microbiol	2023	Clinical utility of metagenomic next-generation sequencing in pathogen detection for lower respiratory tract infections and impact on clinical outcomes in southernmost China	[12]
38264726	Wang JZ et al.	Front Cell Infect Microbiol	2024	Etiology of lower respiratory tract in pneumonia based on metagenomic next-generation sequencing: a retrospective study	[13]
35908723	Liang M et al.	Int J Infect Dis	2022	Metagenomic next-generation sequencing for accurate diagnosis and management of lower respiratory tract infections	[14]
35724965	Li H et al.	Clin Respir J	2022	Metagenomic next-generation sequencing for the diagnosis of Chlamydia psittaci pneumonia	[15]
37871727	Zhao H et al.	J Microbiol Methods	2023	Metagenomic next-generation sequencing of bronchoalveolar lavage fluid in non-severe and severe pneumonia patients	[16]
35622934	Li N et al.	J Clin Lab Anal	2022	Clinical application of metagenomic next-generation sequencing technology in the diagnosis and treatment of pulmonary infection pathogens: A prospective single-center study of 138 patients	[17]
35847093	Zhao D et al.	Front Microbiol	2022	Diagnostic Value and Clinical Application of mNGS for Post-Liver Transplantation Infection: A Cross-Sectional Study With Case Reports	[18]
38368306	Lv T et al.	Infection	2024	Utilizing metagenomic next-generation sequencing for pathogen detection and diagnosis in lower respiratory tract infections in real-world clinical practice	[19]

38997717	Zhang Z et al.	BMC Pulm Med	2024	Metagenomic next-generation sequencing promotes diagnosis and treatment of Pneumocystis jirovecii pneumonia in non-HIV infected children: a retrospective study	[20]
36211959	Zhang B et al.	Front Cell Infect Microbiol	2022	Comparing the application of mNGS after combined pneumonia in hematologic patients receiving hematopoietic stem cell transplantation and chemotherapy: A retrospective analysis	[21]
32879643	Chen X et al.	Can J Infect Dis Med Microbiol	2020	Blood and Bronchoalveolar Lavage Fluid Metagenomic Next-Generation Sequencing in Pneumonia	[22]
35979088	Bao S et al.	Front Cell Infect Microbiol	2022	Metagenomic next-generation sequencing for the diagnosis of pulmonary aspergillosis in non-neutropenic patients: a retrospective study	[23]
37457591	Deng Z et al.	Front Med (Lausanne)	2023	BALF metagenomic next-generation sequencing analysis in hematological malignancy patients with suspected pulmonary infection: clinical significance of negative results	[24]
35837477	Wang D et al.	Front Cell Infect Microbiol	2022	Metagenomic Next-Generation Sequencing Successfully Detects Pulmonary Infectious Pathogens in Children With Hematologic Malignancy	[25]
36277011	Wang L et al.	Comput Math Methods Med	2022	The Value of Macrogene Second-Generation Sequencing in the Diagnosis, Guidance of Drug Use, and Efficacy Monitoring of Infectious Pneumonia in Premature Infants	[26]
34790588	Zhao Z et al.	Front Cell Infect Microbiol	2021	Prevalence of Fungal and Bacterial Co-Infection in Pulmonary Fungal Infections: A Metagenomic Next Generation Sequencing-Based Study	[27]
34197923	Zhou H et al.	J Mol Diagn	2021	Clinical Impact of Metagenomic Next-Generation Sequencing of Bronchoalveolar Lavage in the Diagnosis and Management of Pneumonia: A Multicenter Prospective Observational Study	[28]
36034706	Zhao YC et al.	Front Cell Infect Microbiol	2022	Role and Clinical Application of Metagenomic Next-Generation Sequencing in Immunocompromised Patients With Acute Respiratory Failure During Veno-Venous Extracorporeal Membrane Oxygenation	[29]
38914949	Liu M et al.	BMC Infect Dis	2024	The etiological diagnostic value of metagenomic next-generation sequencing in suspected community-acquired pneumonia	[30]
37506257	Yan M et al.	J Infect Dis	2024	Impact of Metagenomic Next-Generation Sequencing of Bronchoalveolar Lavage Fluid on Antimicrobial Stewardship in Patients With Lower Respiratory Tract Infections: A Retrospective Cohort Study	[31]
38561853	Chen H et al.	Eur J Med Res	2024	Assessment and clinical utility of metagenomic next-generation sequencing for suspected lower respiratory tract infections	[32]
39067508	Wu X et al.	Chest	2024	Effect of Metagenomic Next-Generation Sequencing on Clinical Outcomes of Patients With Severe Community-Acquired Pneumonia in the ICU: A Multicenter, Randomized Controlled Trial	[33]
35783441	Wang D et al.	Front Microbiol	2022	Metagenomic Next-Generation Sequencing Is Highly Efficient in Diagnosing Pneumocystis Jirovecii Pneumonia in the Immunocompromised Patients	[34]
34141628	Sun T et al.	Front Cell Infect Microbiol	2021	Metagenomic Next-Generation Sequencing for Pathogenic Diagnosis and Antibiotic Management of Severe Community-Acquired Pneumonia in Immunocompromised Adults	[35]
37900317	Li XX et al.	Front Cell Infect Microbiol	2023	Clinical application of metagenomic next-generation sequencing in non-immunocompromised patients with severe pneumonia supported by veno-venous extracorporeal membrane oxygenation	[36]
31813078	Li Y et al.	Eur J Clin Microbiol Infect Dis	2020	Application of metagenomic next-generation sequencing for bronchoalveolar lavage diagnostics in critically ill patients	[37]
36590589	Li S et al.	Front Cell Infect Microbiol	2022	The clinical significance of in-house metagenomic next-generation sequencing for bronchoalveolar lavage fluid diagnostics in patients with lower respiratory tract infections	[38]
39210307	Zheng Y et al.	BMC Pulm Med	2024	Clinical utility of metagenomic next-generation sequencing on bronchoalveolar lavage fluid in diagnosis of lower respiratory tract infections	[39]
37938162	Charalampous T et al.	Am J Respir Crit Care Med	2024	Routine Metagenomics Service for ICU Patients with Respiratory Infection	[40]

37663088	Zhang H et al.	Open Forum Infect Dis	2023	The Utility of Metagenomic Next-Generation Sequencing (mNGS) in the Management of Patients With Bronchiectasis: A Single-Center Retrospective Study of 93 Cases	[41]
36389134	Ma W et al.	Front Cell Infect Microbiol	2022	Negative results of bronchoalveolar lavage fluid metagenomic next-generation sequencing in critically ill patients	[42]
38455752	Chen XH et al.	Transl Pediatr	2024	Application value of metagenomics next-generation sequencing in the diagnosis of respiratory virus infection after congenital heart surgery	[43]
38993672	Jing C et al.	iScience	2024	Optimizing treatment administration strategies using negative mNGS results in corticosteroid-sensitive diffuse parenchymal lung diseases	[44]
35221789	Ju CR et al.	Transpl Int	2022	Metagenomic Next-Generation Sequencing for Diagnosing Infections in Lung Transplant Recipients: A Retrospective Study	[45]
37272100	Luo W et al.	Discov Med	2023	Comparison of Third-Generation Sequencing Technology and Traditional Microbiological Detection in Pathogen Diagnosis of Lower Respiratory Tract Infection	[46]
38351916	Yao A et al.	Front Microbiol	2024	Higher diagnostic value of metagenomic next-generation sequencing in acute infection than chronic infection: a multicenter retrospective study	[47]
36743335	Dong Y et al.	Infect Drug Resist	2023	Advancing Microbe Detection for Lower Respiratory Tract Infection Diagnosis and Management with Metagenomic Next-Generation Sequencing	[48]
37529954	Zhong S et al.	Chinese Journal of Contemporary Pediatrics	2023	[Value of metagenomic next-generation sequencing in children with hematological malignancies complicated with infections]	[49]
36124109	Fu Y et al.	Infect Drug Resist	2022	Metagenomic Next-Generation Sequencing in the Diagnosis of Infectious Fever During Myelosuppression Among Pediatric Patients with Hematological and Neoplastic Diseases	[50]
38112143	Feng RG et al.	Zhongguo Dang Dai Er Ke Za Zhi	2023	[Application of metagenomic next-generation sequencing of bronchoalveolar lavage fluid in the diagnosis and treatment of refractory pneumonia in children]	[51]
39045107	Luo W et al.	Infect Drug Resist	2024	Metagenomic Next-Generation Sequencing for Accurate Diagnosis of Pneumocystis jirovecii Pneumonia: A Comparative Study with Traditional Methods	[52]
37408612	Sun H et al.	Front Cell Infect Microbiol	2023	Combination of transbronchial cryobiopsy based clinic-radiologic-pathologic strategy and metagenomic next-generation sequencing for differential diagnosis of rapidly progressive diffuse parenchymal lung diseases	[53]

6 Supplementary Table 2: Candidate consensus statements and the results from Delphi round one and two.

Statement	Agreement %	Median Likert (1–5)	IQR	Result
ROUND 1				
Q1 Thresholds for reporting detections of micro-organisms should be based on evidence from validation studies against standard-of-care diagnostics.	90	4	1	Accept
Q2 Obligate pathogens (e.g. *Mycobacterium tuberculosis*) should be reported whenever detected, regardless of abundance.	84	5	1	Accept
Q3 Metagenomic detections of organisms below pre-specified thresholds should not be reported.	63	4	1	Reject
Q4 Organisms present in negative controls and patient samples at comparable levels should not be reported.	90	5	1	Accept
Q5 Detections of organisms on a predefined respiratory-pathogen list that meet reporting thresholds should be reported directly, whereas detections of others should undergo additional clinical adjudication.	63	4	1	Reject
Q6 Reporting decisions may rely on expert clinical adjudication rather than fixed numeric thresholds, particularly where quantitative validation is unfeasible (e.g. rare organisms).	95	4	1	Accept
Q7 Reports may summarise mixed oral flora collectively rather than listing each species.	100	5	1	Accept
Q8 Detections of potential pathogens (e.g. *S. pneumoniae*, *H. influenzae*, *S. dysgalactiae*) above threshold but amongst oral flora should be available to the validating clinician.	90	5	1	Accept
Q9 The clinician validating results should consider specimen type, relative abundance, and clinical context before deciding whether to report potential pathogens amongst oral flora.	95	5	0.5	Accept
Q10 *Streptococcus pyogenes* should always be reported when detected in respiratory samples.	95	4	0.5	Accept
Q11 *Candida* sp. detected in respiratory samples should be reported when found above threshold.	47	3.5	1	Reject
Q12 Detections of *Tropheryma whipplei* may be suppressed or considered part of oral flora.	47	4	1	Reject
Q13 Detections of pathogens with infection-control / public-health importance (e.g. *M. tuberculosis*, *Legionella*) above threshold should be notified to infection control and/or public health.	90	5	1	Accept
Q14 When detected in sputum samples, anaerobes should be reported as oral flora or commensals.	53	4	1	Reject
Q15 Where a mixture of anaerobes with non-anaerobic commensal bacteria is present, this could be reported as “oral flora including anaerobes.”	84	4	1	Accept
Q16 When only anaerobes are detected, “Anaerobes detected” may be reported.	84	4	1	Accept
Q17 Species of anaerobes detected in pleural fluids should be named at the genus or species level.	74	4	1	Accept
Q18 Detections of herpesviruses should be assessed by the clinician validating results.	95	4	1	Accept
Q19 Detections should be interpreted with consideration of the patient’s immune status and clinical context.	90	5	1	Accept

Q20 Detections judged to be reactivations can be suppressed and not released to clinicians outside the infection team.	58	4	2	Reject
Q21 Special populations (e.g. allogeneic HSCT recipients and neonates) may require different reporting rules compared to the general ICU population.	79	4	1	Accept
Q22 Where clinical significance is suspected, the diagnosis should always be confirmed with additional testing (e.g. viral load, serology, cytopathology).	79	4	1	Accept
Q23 Where herpesviruses are reported, an interpretative comment should be added (e.g. “Detection of herpesviruses is common in critically unwell patients and likely reflects reactivation; please correlate clinically”).	95	4	1	Accept
Q24 Detections of *Pneumocystis jirovecii* above threshold should be reported in lower respiratory-tract samples.	90	5	1	Accept
Q25 Detections of *Pneumocystis jirovecii* above threshold should be reported in all respiratory sample types.	58	4	1	Reject
Q26 Reporting of *Pneumocystis jirovecii* should be accompanied by an interpretative comment noting potential colonisation.	95	4	1	Accept
Q27 Genotypic predictions of antimicrobial resistance may be reported if they have been formally validated.	100	4	1	Accept
ROUND 2				
Q1 Trophyerma whipplei should be assessed at clinical validation and if judged to be clinically insignificant, it may be suppressed or considered part of oral flora	100 %	4.0	0.0	Accept
Q2 Detections of PJP in upper respiratory tract samples should be assessed at clinical validation and may be reported if considered potentially clinically significant.	100%	4.0	0.0	Accept
Q3 When anaerobes are predominant amongst oral flora, they may be reported as oral flora with predominant anaerobes	93%	4	0.0	Accept
Q4 Detections of organisms on a pre-defined respiratory pathogen list that meet reporting thresholds should be reported directly, whereas detections of other pathogens should be assessed further at clinical validation.	93%	4	0.0	Accept
Q5 Noting that thresholds may differ for obligate and opportunistic pathogens, detections of organisms below the pre-specified thresholds should not be reported.	86%	4	0.0	Accept
Q6 Candida sp. detected in respiratory samples should be reported when found above threshold in respiratory samples	79%	4	0.0	Accept
Q7 Detections of herpesviruses can be suppressed and not released to clinicians outside the infection team if judged to be reactivation at clinical validation.	36%	3	2.0	Reject
ROUND 3				
Q1 Herpesviruses can be suppressed and not released to clinicians outside the infection team if judged to be non-significant at clinical validation'	80%	4	1.0	Accept

Risk of bias and applicability

No study was judged to be at low risk of bias across all four domains, and only 2/53 studies had three domains at low risk (Supplementary Figure 1).

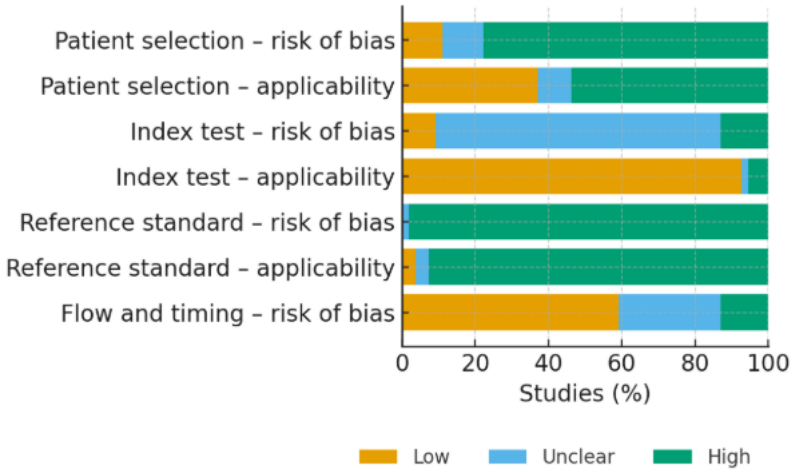
For patient selection, only 6/53 (11%) studies were at low risk of bias, whereas 41/53 (77%) were judged high risk and 6/53 (11%) unclear. Consecutive or random enrolment was explicitly reported in just 6/53 (11%) studies, with most either clearly non-consecutive (8/53, 15%) or not described (39/53, 74%). About half of studies (27/53, 51%) were judged to have inappropriate exclusions, despite most avoiding a classic case-control design (51/53, 96%). Applicability concerns for patient selection were also frequent: 28/53 (53%) studies were judged high concern and only 20/53 (38%) low concern, reflecting that many cohorts did not clearly match the intended target population or setting.

Risk of bias for the index test domain was dominated by unclear judgements, with 43/53 (81%) rated as unclear. Blinding was rarely documented: index test results were clearly interpreted without knowledge of the reference standard in only 2/53 (4%) studies, with 12/53 (23%) explicitly unblinded and 39/53 (74%) unclear. Thresholds for calling metagenomic detections were reported as pre-specified in 37/53 (70%) studies, but remained unclear in 16/53 (30%). In contrast, concerns about applicability of the index test were generally low: 51/53 (96%) studies were judged low concern and only 1/53 (2%) high concern, with 1/53 (2%) unclear, indicating that the way respiratory metagenomics was performed and interpreted usually aligned with the review question.

The reference-standard domain showed the most consistent problems. Fifty-two of 53 (98%) studies were judged at high risk of bias and the remaining one was unclear; none were rated low risk. In most cohorts, the reference standard consisted of heterogeneous composite criteria, typically combining routine microbiology (culture ± targeted PCR) with post-hoc clinical adjudication. This likely reflects the difficulty with diagnosing respiratory infection, especially in critically ill patients. Adjudication panels or treating teams were frequently aware of the metagenomic findings, so the index test contributed directly to the final classification of infection versus colonisation or contamination, introducing incorporation and diagnostic review bias. Verification was also non-uniform: not all participants received the same set of reference tests, and fungal or viral investigations were often ordered selectively according to clinical suspicion. Only 2/53 (4%) studies were judged to use a reference standard likely to correctly classify the target condition, whereas 37/53 (70%) were judged “No” and 14/53 (26%) “Unclear”. Timing and sample type for reference tests commonly differed from those used for metagenomics (for example, sputum versus BAL or investigations performed days apart), making discrepancies difficult to interpret. Blinding was again uncommon: reference standard results were interpreted without knowledge of the metagenomic result in only 4/53 (8%) studies, with 44/53 (83%) explicitly unblinded and 5/53 (9%) unclear. Applicability concerns for the reference standard mirrored this pattern: 49/53 (92%) studies were judged high concern and only 2/53 (4%) low concern, with 2/53 (4%) unclear, reflecting that many truth sets focused on bacterial culture in selected populations, used non-standard or incomplete testing for viruses and fungi, and relied on locally defined adjudication criteria that do not fully align with our review question on pan-microbial, clinically significant respiratory infection.

Flow and timing were comparatively better reported. Thirty-three of 53 (62%) studies were judged at low risk of bias in this domain, 5/53 (9%) high risk, and 15/53 (28%) unclear. Most studies had an appropriate interval between metagenomic testing and the reference standard (48/53, 91%) and included all patients in the analysis (47/53, 89%). However, not all patients consistently received a reference standard (7/53, 13%), and 17/53 (32%) studies used different reference standards within the same cohort, contributing to high or unclear risk of bias for flow and timing in a substantial minority of studies.

Supplementary Figure 1: Risk of bias and applicability across QUADAS-2 domains.



References

1. Jin X, Li J, Shao M, Lv X, Ji N, Zhu Y, et al. Improving Suspected Pulmonary Infection Diagnosis by Bronchoalveolar Lavage Fluid Metagenomic Next-Generation Sequencing: a Multicenter Retrospective Study. Microbiol Spectr. American Society for Microbiology; 2022;10. https://doi.org/10.1128/spectrum.02473-21

2. Xie F, Duan Z, Zeng W, Xie S, Xie M, Fu H, et al. Clinical metagenomics assessments improve diagnosis and outcomes in community-acquired pneumonia. BMC Infect Dis. BioMed Central Ltd; 2021;21. https://doi.org/10.1186/s12879-021-06039-1

3. Liu Y, Zhang R, Yao B, Yang J, Ge H, Zheng S, et al. Metagenomics next-generation sequencing provides insights into the causative pathogens from critically ill patients with pneumonia and improves treatment strategies. Front Cell Infect Microbiol. Frontiers Media S.A.; 2023;12. https://doi.org/10.3389/fcimb.2022.1094518

4. Charalampous T, Alcolea-Medina A, Snell LB, Williams TGS, Batra R, Alder C, et al. Evaluating the potential for respiratory metagenomics to improve treatment of secondary infection and detection of nosocomial transmission on expanded COVID-19 intensive care units. Genome Med. 2021;13:182. https://doi.org/10.1186/s13073-021-00991-y

5. Zhang P, Chen Y, Li S, Li C, Zhang S, Zheng W, et al. Metagenomic next-generation sequencing for the clinical diagnosis and prognosis of acute respiratory distress syndrome caused by severe pneumonia: A retrospective study. PeerJ. PeerJ Inc.; 2020;8. https://doi.org/10.7717/peerj.9623

6. Yang F, Li J, Qi B, Zou L, Shi Z, Lei Y, et al. Clinical Symptoms and Outcomes of Severe Pneumonia Caused by Chlamydia psittaci in Southwest China. Front Cell Infect Microbiol. Frontiers Media S.A.; 2022;11. https://doi.org/10.3389/fcimb.2021.727594

7. Li X, Xiao T, Hu P, Yan K, Wu J, Tu X, et al. Clinical, radiological and pathological characteristics of moderate to fulminant psittacosis pneumonia. PLoS One. Public Library of Science; 2022;17. https://doi.org/10.1371/journal.pone.0270896

8. Tang X, Wang N, Liu G, Tan H, Li AM, Gao YQ, et al. Psittacosis caused severe community-acquired pneumonia accompanied by acute hypoxic respiratory failure: a multicenter retrospective cohort study from China. BMC Infect Dis. BioMed Central Ltd; 2023;23. https://doi.org/10.1186/s12879-023-08283-z

9. Zhan Y, Xu T, He F, Guan WJ, Li Z, Li S, et al. Clinical Evaluation of a Metagenomics-Based Assay for Pneumonia Management. Front Microbiol. Frontiers Media S.A.; 2021;12. https://doi.org/10.3389/fmicb.2021.751073

10. Chen L, Zhao Y, Wei J, Huang W, Ma Y, Yang X, et al. Metagenomic Next-Generation Sequencing for the Diagnosis of Neonatal Infectious Diseases. Microbiol Spectr. American Society for Microbiology; 2022;10. https://doi.org/10.1128/spectrum.01195-22

11. Yin Q, Li Y, Pan H, Hui T, Yu Z, Wu H, et al. Atypical pneumonia caused by Chlamydia psittaci during the COVID-19 pandemic. International Journal of Infectious Diseases. Elsevier B.V.; 2022;122:622–7. https://doi.org/10.1016/j.ijid.2022.07.027

12. Hao J, Li W, Wang Y, Zhao J, Chen Y. Clinical utility of metagenomic next-generation sequencing in pathogen detection for lower respiratory tract infections and impact on clinical outcomes in southernmost

China. Front Cell Infect Microbiol. Frontiers Media SA; 2023;13. https://doi.org/10.3389/fcimb.2023.1271952

13. Wang JZ, Yuan D, Yang XH, Sun CH, Hou LL, Zhang Y, et al. Etiology of lower respiratory tract in pneumonia based on metagenomic next-generation sequencing: a retrospective study. Front Cell Infect Microbiol. Frontiers Media SA; 2023;13. https://doi.org/10.3389/fcimb.2023.1291980

14. Liang M, Fan Y, Zhang D, Yang L, Wang X, Wang S, et al. Metagenomic next-generation sequencing for accurate diagnosis and management of lower respiratory tract infections. Int J Infect Dis. 2022;122:921–9. https://doi.org/10.1016/j.ijid.2022.07.060

15. Li H, Hao B, Wang Y, Yu D, Chen Z, Du D, et al. Metagenomic next-generation sequencing for the diagnosis of Chlamydia psittaci pneumonia. Clinical Respiratory Journal. John Wiley and Sons Inc; 2022;16:513–21. https://doi.org/10.1111/crj.13519

16. Zhao H, Zhao Y, Yan N, Wang Y, Li W, Zhao J, et al. Metagenomic next-generation sequencing of bronchoalveolar lavage fluid in non-severe and severe pneumonia patients. J Microbiol Methods. Elsevier B.V.; 2023;215. https://doi.org/10.1016/j.mimet.2023.106848

17. Li N, Ma X, Zhou J, Deng J, Gu C, Fei C, et al. Clinical application of metagenomic next-generation sequencing technology in the diagnosis and treatment of pulmonary infection pathogens: A prospective single-center study of 138 patients. J Clin Lab Anal. John Wiley and Sons Inc; 2022;36. https://doi.org/10.1002/jcla.24498

18. Zhao D, Guo L, Lian D, Gu Y, Yan X, Hu H, et al. Diagnostic Value and Clinical Application of mNGS for Post-Liver Transplantation Infection: A Cross-Sectional Study With Case Reports. Front Microbiol. Frontiers Media S.A.; 2022;13. https://doi.org/10.3389/fmicb.2022.919363

19. Lv T, Zhao Q, Liu J, Wang S, Wu W, Miao L, et al. Utilizing metagenomic next-generation sequencing for pathogen detection and diagnosis in lower respiratory tract infections in real-world clinical practice. Infection. Springer Science and Business Media Deutschland GmbH; 2024;52:625–36. https://doi.org/10.1007/s15010-024-02185-1

20. Zhang Z, Liu T, Ming M, Shen M, Zhang Y, Chen H, et al. Metagenomic next-generation sequencing promotes diagnosis and treatment of Pneumocystis jirovecii pneumonia in non-HIV infected children: a retrospective study. BMC Pulm Med. BioMed Central Ltd; 2024;24. https://doi.org/10.1186/s12890-024-03135-1

21. Zhang B, Gui R, Wang Q, Jiao X, Li Z, Wang J, et al. Comparing the application of mNGS after combined pneumonia in hematologic patients receiving hematopoietic stem cell transplantation and chemotherapy: A retrospective analysis. Front Cell Infect Microbiol. Frontiers Media S.A.; 2022;12. https://doi.org/10.3389/fcimb.2022.969126

22. Chen X, Ding S, Lei C, Qin J, Guo T, Yang D, et al. Blood and Bronchoalveolar Lavage Fluid Metagenomic Next-Generation Sequencing in Pneumonia. Canadian Journal of Infectious Diseases and Medical Microbiology. Hindawi Limited; 2020;2020. https://doi.org/10.1155/2020/6839103

23. Bao S, Song H, Chen Y, Zhong C, Tang H. Metagenomic next-generation sequencing for the diagnosis of pulmonary aspergillosis in non-neutropenic patients: a retrospective study. Front Cell Infect Microbiol. Frontiers Media S.A.; 2022;12. https://doi.org/10.3389/fcimb.2022.925982

24. Deng Z, Tang Y, Tu Y, Liu M, Cheng Q, Zhang J, et al. BALF metagenomic next-generation sequencing analysis in hematological malignancy patients with suspected pulmonary infection: clinical significance of negative results. Front Med (Lausanne). Frontiers Media SA; 2023;10. https://doi.org/10.3389/fmed.2023.1195629

25. Wang D, Wang W, Ding Y, Tang M, Zhang L, Chen J, et al. Metagenomic Next-Generation Sequencing Successfully Detects Pulmonary Infectious Pathogens in Children With Hematologic Malignancy. Front Cell Infect Microbiol. Frontiers Media S.A.; 2022;12. https://doi.org/10.3389/fcimb.2022.899028

26. Wang L, Zha P, Wang Y, Kong Y, Su Y, Dai L, et al. The Value of Macrogene Second-Generation Sequencing in the Diagnosis, Guidance of Drug Use, and Efficacy Monitoring of Infectious Pneumonia in Premature Infants. Comput Math Methods Med. Hindawi Limited; 2022;2022. https://doi.org/10.1155/2022/4398614

27. Zhao Z, Song J, Yang C, Yang L, Chen J, Li X, et al. Prevalence of Fungal and Bacterial Co-Infection in Pulmonary Fungal Infections: A Metagenomic Next Generation Sequencing-Based Study. Front Cell Infect Microbiol. Frontiers Media S.A.; 2021;11. https://doi.org/10.3389/fcimb.2021.749905

28. Zhou H, Larkin PMK, Zhao D, Ma Q, Yao Y, Wu X, et al. Clinical Impact of Metagenomic Next-Generation Sequencing of Bronchoalveolar Lavage in the Diagnosis and Management of Pneumonia: A Multicenter Prospective Observational Study. Journal of Molecular Diagnostics. Elsevier B.V.; 2021;23:1259–68. https://doi.org/10.1016/j.jmoldx.2021.06.007

29. Zhao YC, Ding YZ, Zhao X, Fu GW, Huang MJ, Li XX, et al. Role and Clinical Application of Metagenomic Next-Generation Sequencing in Immunocompromised Patients With Acute Respiratory Failure During Veno-Venous Extracorporeal Membrane Oxygenation. Front Cell Infect Microbiol. Frontiers Media S.A.; 2022;12. https://doi.org/10.3389/fcimb.2022.877205

30. Liu M, Zhang H, Li L, Mao J, Li R, Yin J, et al. The etiological diagnostic value of metagenomic next-generation sequencing in suspected community-acquired pneumonia. BMC Infect Dis. BioMed Central Ltd; 2024;24. https://doi.org/10.1186/s12879-024-09507-6

31. Yan M, Zou X, Wang Y, Wang C, Wang Y, Liu Z, et al. Impact of Metagenomic Next-Generation Sequencing of Bronchoalveolar Lavage Fluid on Antimicrobial Stewardship in Patients With Lower Respiratory Tract Infections: A Retrospective Cohort Study. Journal of Infectious Diseases. Oxford University Press; 2024;229:223–31. https://doi.org/10.1093/infdis/jiad296

32. Chen H, Huang Q, Wu W, Wang Z, Wang W, Liu Y, et al. Assessment and clinical utility of metagenomic next-generation sequencing for suspected lower respiratory tract infections. Eur J Med Res. BioMed Central Ltd; 2024;29. https://doi.org/10.1186/s40001-024-01806-7

33. Wu X, Sun T, He H, Xing L, Cheng Z, Geng S, et al. Effect of Metagenomic Next-Generation Sequencing on Clinical Outcomes of Patients With Severe Community-Acquired Pneumonia in the ICU: A Multicenter, Randomized Controlled Trial. Chest. Elsevier Inc.; 2025;167:362–73. https://doi.org/10.1016/j.chest.2024.07.144

34. Wang D, Fang S, Hu X, Xu Q, Chu X, Mei X, et al. Metagenomic Next-Generation Sequencing Is Highly Efficient in Diagnosing Pneumocystis Jirovecii Pneumonia in the Immunocompromised Patients. Front Microbiol. Frontiers Media S.A.; 2022;13. https://doi.org/10.3389/fmicb.2022.913405

35. Sun T, Wu X, Cai Y, Zhai T, Huang L, Zhang Y, et al. Metagenomic Next-Generation Sequencing for Pathogenic Diagnosis and Antibiotic Management of Severe Community-Acquired Pneumonia in Immunocompromised Adults. Front Cell Infect Microbiol. Frontiers Media S.A.; 2021;11. https://doi.org/10.3389/fcimb.2021.661589

36. Li XX, Niu CZ, Zhao YC, Fu GW, Zhao H, Huang MJ, et al. Clinical application of metagenomic next-generation sequencing in non-immunocompromised patients with severe pneumonia supported by veno-venous extracorporeal membrane oxygenation. Front Cell Infect Microbiol. Frontiers Media SA; 2023;13. https://doi.org/10.3389/fcimb.2023.1269853

37. Li Y, Sun B, Tang X, Liu Y lan, He H yong, Li X yan, et al. Application of metagenomic next-generation sequencing for bronchoalveolar lavage diagnostics in critically ill patients. European Journal of Clinical Microbiology and Infectious Diseases. Springer; 2020;39:369–74. https://doi.org/10.1007/s10096-019-03734-5

38. Li S, Qin J, Zhou P, Peng M, Qian J, Cai Y, et al. The clinical significance of in-house metagenomic next-generation sequencing for bronchoalveolar lavage fluid diagnostics in patients with lower respiratory tract infections. Front Cell Infect Microbiol. Frontiers Media S.A.; 2022;12. https://doi.org/10.3389/fcimb.2022.961746

39. Zheng Y, Liu W, Xiao T, Chen H, Liu Y. Clinical utility of metagenomic next-generation sequencing on bronchoalveolar lavage fluid in diagnosis of lower respiratory tract infections. BMC Pulm Med. BioMed Central Ltd; 2024;24. https://doi.org/10.1186/s12890-024-03237-w

40. Charalampous T, Alcolea-Medina A, Snell LB, Alder C, Tan M, Williams TGS, et al. Routine Metagenomics Service for ICU Patients with Respiratory Infection. Am J Respir Crit Care Med. American Thoracic Society; 2024;209:164–74. https://doi.org/10.1164/rccm.202305-0901OC

41. Zhang H, Shen D, Zhou J, Yang Q, Ying Y, Li N, et al. The Utility of Metagenomic Next-Generation Sequencing (mNGS) in the Management of Patients with Bronchiectasis: A Single-Center Retrospective Study of 93 Cases. Open Forum Infect Dis. Oxford University Press; 2023;10. https://doi.org/10.1093/ofid/ofad425

42. Ma W, Zhao Y, Lu X, Zhang L, Ma X, Gao J, et al. Negative results of bronchoalveolar lavage fluid metagenomic next-generation sequencing in critically ill patients. Front Cell Infect Microbiol. Frontiers Media S.A.; 2022;12. https://doi.org/10.3389/fcimb.2022.962283

43. Chen XH, Zhou SJ, Liu YY, Cao H, Zheng YR, Chen Q. Application value of metagenomics next-generation sequencing in the diagnosis of respiratory virus infection after congenital heart surgery. Transl Pediatr. AME Publishing Company; 2024;13:260–70. https://doi.org/10.21037/tp-23-341

44. Jing C, Ding Y, Zhou J, Zhang Q, Wang M, Ou Q, et al. Optimizing treatment administration strategies using negative mNGS results in corticosteroid-sensitive diffuse parenchymal lung diseases. iScience. Elsevier Inc.; 2024;27. https://doi.org/10.1016/j.isci.2024.110218

45. Ju C-R, Lian Q-Y, Guan W-J, Chen A, Zhang J-H, Xu X, et al. Metagenomic Next-Generation Sequencing for Diagnosing Infections in Lung Transplant Recipients: A Retrospective Study. Transplant International. Frontiers Media SA; 2022;36. https://doi.org/10.3389/ti.2022.10265

46. Luo W, He Y, Xu J, Zhang S, Li C, Lv J, et al. Comparison of Third-Generation Sequencing Technology and Traditional Microbiological Detection in Pathogen Diagnosis of Lower Respiratory Tract Infection. Discov Med. Discovery Medicine; 2023;35:332. https://doi.org/10.24976/discov.med.202335176.34

47. Yao A, Wang J, Xu Q, Shah BK, Sun K, Hu F, et al. Higher diagnostic value of metagenomic next-generation sequencing in acute infection than chronic infection: a multicenter retrospective study. Front Microbiol. Frontiers Media SA; 2024;15. https://doi.org/10.3389/fmicb.2024.1295184

48. Dong Y, Chen Q, Tian B, Li J, Li J, Hu Z. Advancing Microbe Detection for Lower Respiratory Tract Infection Diagnosis and Management with Metagenomic Next-Generation Sequencing. Infect Drug Resist. Dove Medical Press Ltd; 2023;16:677–94. https://doi.org/10.2147/IDR.S387134

49. Zhong S, Yang MH. Value of metagenomic next-generation sequencing in children with hematological malignancies complicated with infections. Chinese Journal of Contemporary Pediatrics. Central South University; 2023;25:718–25. https://doi.org/10.7499/j.issn.1008-8830.2212059

50. Fu Y, Zhu X, Cao P, Shen C, Qian X, Miao H, et al. Metagenomic Next-Generation Sequencing in the Diagnosis of Infectious Fever During Myelosuppression Among Pediatric Patients with Hematological and Neoplastic Diseases. Infect Drug Resist. 2022;Volume 15:5425–34. https://doi.org/10.2147/IDR.S379582

51. Feng RG, Zhou LY, Dou R, Zhou XY, Wang LR, Han LH, et al. Application of metagenomic next-generation sequencing of bronchoalveolar lavage fluid in the diagnosis and treatment of refractory pneumonia in children. Chinese Journal of Contemporary Pediatrics. Central South University; 2023;25:1253–8. https://doi.org/10.7499/j.issn.1008-8830.2307106

52. Luo W, Lin X, Chen Y, Luo W, Zhang H. Metagenomic Next-Generation Sequencing for Accurate Diagnosis of Pneumocystis jirovecii Pneumonia: A Comparative Study with Traditional Methods. Infect Drug Resist. 2024;Volume 17:2965–74. https://doi.org/10.2147/IDR.S459722

53. Sun H, Chen R, Li T, Gao J, Gu X, Zhu X, et al. Combination of transbronchial cryobiopsy based clinic-radiologic-pathologic strategy and metagenomic next-generation sequencing for differential diagnosis of rapidly progressive diffuse parenchymal lung diseases. Front Cell Infect Microbiol. Frontiers Media SA; 2023;13. https://doi.org/10.3389/fcimb.2023.1204024